PEDRO HENRIQUE BRUM TOGNI

HABITAT MANIPULATION FOR CONSERVATION BIOLOGICAL CONTROL IN ORGANIC VEGETABLE CROPS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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SUMÁRIO

RESUMO

TOGNI, Pedro Henrique Brum, D.Sc. Universidade Federal de Viçosa, Fevereiro de 2014. **Manipulação do habitat para o controle biológico conservativo em sistemas orgânicos de produção de hortaliças.** Orientadora: Madelaine Venzon. Co-orientadores: Edison Ryoiti Sujii e Angelo Pallini Filho.

O controle biológico é a principal estratégia utilizada na agricultura orgânica para prevenção dos danos causados por artrópodes praga. Para que o controle biológico possa ser favorecido, a manipulação de habitats nos agroecossistemas deve prover recursos e condições adequadas para a conservação e manutenção dos inimigos naturais na paisagem agrícola. Contudo, também é necessário considerar que as interações entre inimigos naturais, pragas e as estratégias de manejo do habitat ocorrem em escalas que variam de milímetros até quilômetros. O objetivo desta tese foi avaliar como o manejo do habitat em diferentes escalas espaciais pode afetar o controle biológico conservativo em sistemas orgânicos de produção de hortaliças. Para isso, esta tese está estruturada em três capítulos, cada um lidando com diferentes tipos de interações entre inimigos naturais e o agroecossistema com foco nos sistemas orgânicos de produção de hortaliças. No Capítulo I foi avaliado o papel de diferentes habitats cultivados (duas áreas com hortaliças) e não cultivados (áreas de pousio e de vegetação nativa) nos padrões de diversidade, composição das comunidades de artrópodes herbívoros e predadores e como a abundância desses grupos varia temporalmente entre habitats. Foi observado que cada tipo de habitat tem um papel diferente na conservação de ambos os grupos funcionais. Além disso, o manejo local do habitat afeta de forma diferente a abundância de predadores e herbívoros ao longo do tempo. Manter diferentes habitats cultivados e não cultivados contribuiu para conservação de predadores na paisagem agrícola e também para a rápida colonização de novos habitats em resposta ao aumento populacional de herbívoros. No Capítulo II, foi testada a hipótese de que propriedades com uma maior diversidade de habitats, maior diversidade vegetal e menor regime de perturbação são mais favoráveis para a conservação e atuação de inimigos naturais da mosca-branca *Bemisia tabaci* (Hemiptera: Aleyrodidae) em cultivos de tomate. As propriedades amostradas foram agrupadas em quatro categorias que variavam desde sistemas convencionais com baixa diversidade vegetal e com a aplicação frequente de inseticidas até sistemas orgânicos muito diversos e com controle natural de pragas. As propriedades mais diversas e com práticas de manejo menos intensivas foram favoráveis à conservação dos inimigos naturais da mosca-branca, resultando em maior mortalidade por controle biológico. A predação foi o fator chave de mortalidade da mosca-branca e a maior diversidade de predadores resultou em maior mortalidade da mosca-branca. Além disso, uma maior diversidade de predadores reduziu a variação das taxas de predação entre as propriedades amostradas, de modo que a predação foi mais constante nas propriedades mais diversas e menos perturbadas. No Capítulo III foi investigado, em laboratório por que o coentro quando em consórcio com o tomateiro atrai predadores generalistas, utilizando *Cycloneda sanguinea* (Coleoptera: Coccinellidae) como modelo. Voláteis de coentro atraíram o predador e aumentaram a atração por voláteis de tomateiros infestados com pulgões. Parte dessa atração pode ser explicada pelo fato do coentro servir como sítio de oviposição para o predador. As larvas que emergiram dos ovos depositados no coentro são capazes de encontrar tomateiros infestados com pulgões nas proximidades. Além disso, as inflorescências do coentro beneficiam a sobrevivência de *C. sanguinea*, mas não afetam sua reprodução. Todos esses fatores contribuem para a atração e retenção deste predador em campo em cultivos de tomate consorciados com coentro. Em conclusão, a conservação e manejo de inimigos naturais em hortaliças orgânicas dependem da adoção de estratégias em diferentes escalas. O primeiro passo deve ser manter essas espécies na propriedade sob manejo orgânico para a colonização de habitats de interesse na paisagem. Contudo, sua funcionalidade em um dado habitat na propriedade depende da diversidade local e do uso de práticas de manejo menos intensivas. Além disso, as interações entre os recursos introduzidos e os inimigos naturais também devem ser consideradas.

ABSTRACT

TOGNI, Pedro Henrique Brum, D.Sc. Universidade Federal de Viçosa, February 2014. **Habitat manipulation for conservation biological control in organic vegetable crops.** Adviser: Madelaine Venzon. Co-advisers: Edison Ryoiti Sujii and Angelo Pallini Filho.

Biological control is the main strategy used in organic farming to prevent arthropod pest damage. To favor biological control, habitat manipulation in agroecosystems should provide suitable resources and conditions for the conservation and maintenance of natural enemies in the farm level. However, it is necessary also to consider that interactions between natural enemies, pests and habitat manipulation strategies occur at scales that vary from millimeters to kilometers. The aim of this thesis was evaluate how habitat management at different spatial scales affects conservation biological control in organic vegetable crop production. This thesis is structured in three chapters, each one dealing with different types of interactions between natural enemies and the agroecosystems, focusing on organic vegetable crop production. In Chapter I it was evaluated the role of different cropped (two areas with vegetables) and non-cropped habitats (fallow areas and native vegetation) on the patterns of diversity and composition of predator and herbivore arthropod communities and how the abundance of these groups vary between habitats through time. It was observed that each habitat has different roles in the conservation of both functional groups. Moreover, the local management of habitats differently affected the abundance of predators and herbivores through time. Maintaining different cropped and non-cropped habitats contributed to the conservation of predators in the farm level and also to a rapid colonization of new habitats in response to the increase in population of herbivores. In Chapter II, it was tested the hypothesis that a high diversity of habitats and vegetation diversity and a reduced disturbance are favorable to the conservation and performance of natural enemies of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in tomato crops. Farms were grouped in four categories that varied from conventional systems with low diversity of vegetation and high input of chemical insecticides to diversified organic systems with natural pest control. The most diversified farms using less intensive management practices were favorable to whitefly natural enemies' conservation, resulting in an increase in whitefly mortality due to biological control. Predation was the key mortality factor of whiteflies and a greater diversity of predators resulted in an increase in whitefly mortality. Furthermore, the diversity of predators reduced the variability in predation rates among farms, thus predation were more constant in diversified farms using less intensive management practices. In Chapter III it was investigated in laboratory why coriander when intercropped with tomato plants attract generalist predators, using *Cycloneda sanguinea* (Coleoptera: Coccinellidae) as model. Volatiles from coriander plants attracted the predator and increased the attraction to volatiles of aphid-infested tomato plants. This attraction was partially because coriander can be used as oviposition sites by this predator. Larvae emerged from eggs deposited on coriander plants were able to find nearby aphid-infested tomato plants. Moreover, coriander flowers benefited the survivorship of *C. sanguinea*, but not reproduction. All these factors contributed to predator attraction in field when tomato plants are intercropped with coriander. In conclusion, the conservation and management of natural enemies in organic vegetable crops rely on the adoption of strategies at different scales. The first step should be maintaining natural enemy species in the farm level to the colonization of habitats in the landscape. Nevertheless, their functinality in a given habitat depend on the local diversity and use of less intensive management practices. Furthermore, the interaction of natural enemies with introduced resources should also be considered.

GENERAL INTRODUCTION

During the 10th Convention of Biological Diversity it was established that conservation efforts should focus on the services that ecosystems can provide to real interests of humans (*e.g.* food, health, water, biological control, pollination) instead of only on the conservation of the taxonomic diversity (Perrings, et al., 2010; Mace et al., 2012). Although anthropocentric, using this perspective it is possible to state that ecosystem services also occur in human-dominated landscapes, such as agricultural areas (Melo et al., 2013). In fact, landscapes worldwide are a mosaic of natural areas interspersed with agricultural areas. For example, Brazil is one of the main producers of food and fiber in the world and at the same time is considered a mega-diverse country with many hotspots of biodiversity (Myers et al., 2000; Ferreira et al., 2012). Furthermore, agriculture expansion is considered one of the main causes of biodiversity loss worldwide, mainly in tropical regions (Cardinale et al., 2012; Ferreira et al., 2012). This contrasting situation between the needs for crop production and biodiversity conservation demands the development of new management strategies based on the ecological interactions in agricultural areas. Overcame this conflict of interests between food production and biodiversity conservation by developing biodiversity-friendly agricultural landscapes could be also an important opportunity to fit Brazil to the targets of biodiversity conservation.

Organic farming is an important starting point to these changes. The less intensive management practices used in crop production tend to benefit the biodiversity and abundance of several taxa including arthropods, mammals, birds, plants and soil microbes (Bengtsson et al., 2005; Hole et al., 2005). Due to the prohibition of using synthetic products in organic systems, farmes should conserve and maintain several ecosystem services (*e.g.* biological control, decomposition) to crop production that are directly related to biodiversity conservation (Power, 2010; Sandhu et al., 2010). Conventional farmers can also use these ecosystem services, but organic farmers are more dependent on their use. In consequence, the economic value of ecosystem services to organic farmers is greater than to the conventional farmers. (Sandhu et al., 2010). The first step to use these valuable ecosystem services is to identify the needs of farmers.

Although the importance of Brazil for agricultural production in the international scenario, about 85% from the total food internally consumed is produced by small farmers (IPD, 2010). These farmers are characterized by the cultivation of a diversity of crops in small farm areas (0.4 ha – 12 ha) and by the employment of familiar labor in the farm management. Many of these farmers are now adopting the organic management system and Brazil is currently the second largest producer of organic food in the world (IPD, 2010). The organic market in Brazil is responsible for an annual amount of R$ 1.2 billion, which 86% is generated by the organic vegetable production, mostly from familiar growers (IPD, 2010). In organic crops, pest management strategies related to habitat manipulation are one of the main limiting factors for crop production (Zehnder et al., 2007). To avoid problems with arthropod pests, farmers need to conserve and maintain the ecosystem service of biological control.

The conservation and maintenance of biological control as an ecosystem service depends directly on the adoption of farm design and cultural practices that favor the local fauna of natural enemies. Therefore, there is a need for understanding multiple ecological interactions between natural enemies and biotic and abiotic components of agroecosystems in order to prevent pest damage (Zehnder et al., 2007). Such interactions can be affected by local factors (*e.g.* habitat heterogeneity, frequency of disturbance) and regional factors (*e.g.* local species pool, dispersion abilities of organisms, habitat connectivity) (Chase and Bengtsson, 2010) that can also be investigated in order to benefit biological control. This implies that the colonization of habitats by natural enemies and their efficiency as mortality factors of pests could be related to the adoption of habitat management strategies at different scales.

Understanding the spatio-temporal dynamics of natural enemies is especially important to annual crops with a short cycle, such as vegetables. In tropical regions, the climatic conditions allow that many vegetable crop species are cultivated during the entire year. Due to the ephemeral nature of vegetables in the field, herbivore and natural enemy communities are constantly subjected to a high intensity of disturbing factors when a crop is harvested and removed. The local populations are constantly dispersing through the landscape and, therefore, their spatial dynamics are closely associated with landscape characteristics (Thies et al., 2003). Maintaining different habitat types in the landscape (*e.g.* different crops, fallow areas, forests) where natural enemies can disperse and use the resources therein in space and time is a key strategy (Tscharntke et al., 2007). When a new crop is established in a given area of the farm the populations of natural enemies can rapidly colonize the new habitat from the adjacent habitats.

It is also necessary to understand how different habitat types in the farm affect the biological control of key pests in the plot level. For example, agroforestry systems (Harterreiten-Souza et al., 2014), strips of non-crop plants (Amaral et al., 2013), flowering plants (Togni et al., 2010a) and non-cropped areas (Thies et al., 2003) are known to attract and maintain several natural enemy species in the farm. But how the diversity of habitats and the vegetational diversity within a farm affect biological control in a given crop was not completely addressed. Moreover, intensive pest management strategies, such as insecticide spraying, can disrupt biological control. For example, Naranjo and Ellsworth (2009) demonstrated that predators only played a role in the mortality of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in cotton fields in USA when combined with selective insecticides. Possibly, farms with a higher diversity of plants and habitats and with low disturbance levels caused by pest management practices can benefit natural enemy species and, consequently biological control. This is a hypothesis that should be explicily tested and the use of mortality factor analysis can be an important tool to a direct measure of the effects of such habitats characteristics on biological control efficiency.

Another important factor that might affect biological control is how natural enemies interact with resources and with other species within a habitat. The provision of different types of resources as well as shelter, suitable microclimatic conditions, oviposition sites and plat-provided food (pollen and nectar) by increasing vegetational diversity can favor the attraction and retention of natural enemies (Andow, 1991; Letourneau et al., 2011). However, this positive effect is not always achieved, indicating that increasing vegetational diversity *per se* is not sufficient for a positive effect on biological control (Letourneau et al., 2011). When manipulating a given habitat by increasing plant diversity, plant traits should be take into account (Winkler et al., 2010). For example, intercropping tomato plants with coriander reduced the colonization of tomato crops by *B. tabaci* (Togni et al., 2009). Coriander odors can mask the odors of tomato plants and then *B. tabaci* tend to avoid areas whith tomato plants intercropped with coriander and move to other habitats (Togni et al., 2010b). At the same time, coriander plants attracted several natural enemy species, mainly coccinellids, during the vegetative and flowering phases (Togni et al., 2010a). In order to better manage this interaction in favor of biological control, it is necessary to fully understand how natural enemies, such as coccinellids, interact with coriander plants.

Based on all this information, it is possible to infer that the interactions between natural enemies, prey and habitat management strategies occur in spatial scales that can vary from millimeters (*e.g.* interspecific interactions) to kilometers (*e.g* interactions with the habitat types within the landscape). Therefore, cropped and non-cropped habitats should have different roles in the conservation and maintenance of natural enemy species at the farm level. Farms with contrasting levels of vegetational diversity, with different habitat types and different disturbance factors probably directly affect the importance of biological control as mortality factors of insect pests. At a more restricted spatial scale understanding how natural enemy species interact with specific resources can also contribute to their attraction and retention in order to benefit biological control. To test these assumptions this thesis is structured in three chapters dealing with factors at different scales which could affect conservation biological control. All the experiments conducted focused on organic farming or in management strategies suitable to be used in organic farms. In all chapters, predator species and their dynamics in tomato crops were used as models to test how different spatial scales could affect biological control in case-specific situations.

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CHAPTER I

Different habitats within farms affect the communities of predaceous and herbivorous arthropods in organic vegetable crops

Abstract

In organic crops, habitat manipulation should focus on providing resources and conditions in space and time to conserve natural enemies. In this way, such cropping system could rely on the ecosystem service of biological control to prevent problems with arthropod pests. The aim of this study was to identify how predator and herbivores communities are affected by different habitat types in space and time in organic systems. During one year, we sampled the herbivorous and predaceous arthropod fauna in four habitat types in organic farms: *i*) tomato and kale crops (main crop); *ii)* vegetable crops surrounding the main crop (neighborhood crops); *iii*) fallow areas; and *iv*) native forests. Fallow areas presented a greater diversity of predators and herbivores; cropped areas intermediate diversity and forest the lowest. Although the diversity and abundance of predaceous and herbivorous arthropod were quite low in forests, our sampling methodology did not assess a significative part of their communities in such habitat (no saturation of rarefaction curves). Community composition of both groups were more similar in the cropped areas, but also shared most species with the fallow areas, indicating that species can disperse between all agricultural habitats, with fallow areas playing a key role in species conservation. The abundance of predators and herbivores was greater and less constant along the year in both cropped areas and lower and more constant in the non cropped areas. Due to this, the colonization and establishment of species qualitatively and quantitatively depends on the local management of such habitats. The increase in abundance of predators is directly affected by the abundance of herbivores, mainly in the cropped areas. Maintaining habitats cropped and non cropped areas in the farm positively affect the conservation of predators in the farm level and contribute to biological control.

Key-words: biodiversity, agroecology, conservation biological control, ecosystem services, farm management

Introduction

The expansion of agriculture is considered one of the main threats to biodiversity worldwide, due to fragmentation of natural habitats (Tilman et al., 2002; Perrings et al. 2011, Cardinale et al., 2012). In fact, all agricultural systems impose a certain level of risk to biodiversity, high diversities of organisms naturally occurring in a given area are substituted by an artificial and more homogeneous landscape (Sujii et al., 2011). However, some of these impacts could be alleviated by designing "biodiversity-friendly" landscapes where the needs for biodiversity conservation meet the needs for food production, especially in tropical regions (Ferreira et al., 2012; Melo et al., 2013). Organic farming is one of these strategies to reduce the impact of agricultural expansion on biodiversity (Hole et al., 2005). That is because the organic farms should conserve and maintain several ecosystem services, such as biological control, decomposition and pollination, for crop production and these services are directly related to biodiversity in the farm (Zhender et al., 2007; Sandhu et al., 2010). Therefore, ecosystem services in organic systems have an overall economic value greater than for growers in the conventional systems (Sandhu et al., 2010).

Specifically, in relation to the use and maintenance of biological control as an ecosystem service in the agricultural landscape, it is necessary the adoption of farm designs and cultural practices that will favor the local fauna of natural enemies. Increasing the vegetational diversity at the plot level was one of the strategies aiming to attract and benefit natural enemies that received much attention in the last decades (Andow, 1991; Letourneau et al., 2011). This is because increasing plant diversity in and around cropped areas results in a higher availability of prey and alternative resources for natural enemies, given suitable conditions for their attraction and retention even when pests are absent (Landis et al. 2000, Gurr et al. 2003). Although this is an important strategy, recent evidences suggest that habitat diversity in the landscape scale directly interferes with the spatial and temporal dynamics of natural enemies and biological control at the plot level (Thies and Tscharntke, 1999; Thies et al., 2003; Veres et al., 2013). For example, about 75% of the studies reviewed by Bianchi et al. (2006) reported that landscape diversity has a positive effect on natural enemy conservation and biological control.

The vegetables cropped in organic system depend essentially on preventive and sustainable methods for pest management, based on understanding the ecological interactions between different components of agroecosystems in order to avoid curative strategies (Zehnder et al., 2007). As vegetables have a short crop cycles, pest control depends on the availability of a pool of natural enemy species in the farm wich can rapidly colonize the cropped areas from adjacent habitats. Furthermore, the natural enemy and herbivore communities are constantly under a high frequency of disturbing factors such as crop management and harvesting. This produces a high turnover of local populations that will be constantly dispersing among habitats in the landscape and thus their spatial dynamic could be strictly associated with the landscape characteristics and habitats therein (Thies et al., 2003). Therefore, maintaining suitable cropped and non-cropped habitats where natural enemies can disperse and be maintained should be an important strategy toavoid natural enemies dispersion to another areas. After re-colonizing a cropped area the community dynamics could become associated with local habitat characteristics such as alternative resources availability, habitat structure, presence of refuges and oviposition sites, wich also can be manipulated (Werling and Gratton, 2010). To understand the temporal and spatial dynamics of natural enemies at the farm-level, it is also necessary to consider the spatio-temporal dynamics of their prey (herbivores) and how both functional groups are associated.

Probably, in organic vegetable crop production the availability of different habitats, including cropped, non-cropped and native vegetation will affect the movement of natural enemies and herbivores at the farm-level. This could contribute to a rapid colonization of a crop of interest by natural enemies and reduce the herbivore populations in a patch dynamics approach. Consequently, conservation of natural enemies in the farm-level could be related to a better management of herbivore populations at different habitat types. To test this hypothesis we evaluated the patterns of diversity and abundance of generalist predator and herbivore arthropod species in four different habitat types in organic farms cropping vegetables. Specifically, we addressed whether predator and herbivores communities varied in species diversity and composition in each habitat and how such habitats affected the patterns of abundance of these functional groups throughout the time.

Material and methods

Study areas

The study was conducted in four small organic farms located in the Brazilian Federal District, Brazil, from March/2012 to February/2013. The region is located in the core of the Cerrado biome, the Brazilian tropical savanna. The Cerrado is the second major biome in Brazil, occupying about one quarter of the total land area in the country and is also considered a hotspot of biodiversity (Myers et al., 2000). The climate of the region is type Cwa semi-humid with a seasonal climate of a dry winter and a hot summer, according the Köppen-Geiger classification. The wet season is usually from October to April, whereas the dry season occurs from May to September. In general, the temperatures range from 22 °C to 27 °C, with an average rainfall of 1,200 mm per year. However, during the dry season, average temperatures vary from 15 °C to 30 °C, the relative humidity can fall below 15%, and the rainfall is < 100 mm per month (Klink and Machado, 2005).

Each farm was at least 20 km distant from each other. All farms practiced organic management for at least six years. They mainly employ family labor in the farm management and crop production. Different strategies of pest control such as mixtures and botanical insecticides that could be done by their own in the farm are used. The most common products applied to pest control were lime sulfur, Bordeaux mixture, chili pepper extract, neem based products, homeopathy and *Trichogramma* spp. The farmers also applied *Bt*-based products from the end of 2012 until February/2013. All these products were applied only when farmers detected pest presence and at least with a 15-30 days interval. Manual and selective weeding of strips of weeds within and surrounding vegetable planting was another strategy also used to attract natural enemies for pest control.

Each farmer cropped at least 16 different types of vegetables. All growers cultivated tomatoes and kale as the main crop through the year. Crops were planted in small plots (450 – 3,000. m^2) and with at least two other vegetable species nearby (neighborhood crops). Vegetable crop areas were always surrounded by windbreaks with at least three different plant strata (usually trees and shrubs) or by agroforests. These windbreaks were interspersed with other crops and were used as barriers between crop and non-crop areas. The most common species of windbreaks were the Mexican sunflower (*Tithonia diversifolia* (Hemsl.)) (Asteraceae), banana (*Musa* spp.) (Musaceae), conilon coffee (*Coffea canephora* Pierre) (Rubiaceae) and leucaena (*Leucaena leucocephala* (Lam)) (Fabaceae). For details on the main plant species used in the agroforestry systems in the region of the Brazilian Federal District see Harterreiten-Souza et al. (2014).

Regarding the non-crop areas, farmers left fallow areas with different sizes (1,000 – 2,000 m²) for cultivation in the subsequent year. The fallow area was dominated by mucuna-beans (*Mucuna* sp.)(Fabaceae), wich is used as cover crop and as a green manure, when incorporated to the soil. Mucuna-beans were interspersed with several species of unmanaged non-crop plants such as elephant grass (*Pennisetum purpureum* Schum.) (Poaceae) and Mexican sunflowers. These areas were maintained unmanaged during the experiment conduction, except in one farm fallow area where sorghum (*Sorghum bicolor* (L.) Moench.) (Poaceae) and black oat (*Avena strigosa* Schreb.) (Poaceae) were used as green manures. Farms also contained fragments of riparian native forest areas along small rivers inside farm boundaries that occupied at least 20% from the farm total area.

Experimental design and sampling

To evaluate the role of habitat management for predator conservation and as potential sources of these insects for tomato and kale crops at the farm-level, we simultaneously sampled four different habitat types (two cropped and two non-cropped) in each farm. The sampled habitats in each farm were *i)* the main crop (tomato and kale); *ii)* neighborhood crops; *iii)* fallow area; and *iv)* native forest. Tomato and kale crops were considered the main crops and they were cultivated during the entire year, as explained above. Moreover, these two crops represent one of the most important cash crops and farmers reported several problems with insect pests on these crops. We also evaluated the neighborhood crops around the main crop because they are the closest area from the main crop that could potentially serve as the immediate source of species or to receive species before the main crop planting or harvesting, respectively. These neighborhood crops were planted at least 50 m distant from the main crop and the areas between crop areas were left with strips of non-crop plants or interspersed with some trees (native or introduced for commercial purposes). As there were at least two different vegetables planted nearby the main crop, we only sampled the nearest neighborhood crop, and evaluated the number of neighborhood crops along the year as well. All farms also had fallow areas positioned 700-1,100 m distant from the cropped areas. The native forest areas were positioned at the boundaries of the farms. In one of the sampled farms, the native vegetation was used for recreational purposes by the people in the region and this habitat was not sampled in this farm.

Arthropod sampling was done with Malaise traps placed in the core of each habitat type (one trap per habitat) and by direct sampling of insects over the plants within each habitat (Duelli et al., 1999; Russo et al., 2011). Malaise traps were used because this is a standardized passive method that trapped mostly active flyers that fly upward when their flight are obstructed, forcing the insects into a collecting jar in the top of the trap (Duelli et al., 1999). Thus, we could measure patterns of diversity and abundance mainly of active predator and herbivore species that could potentially move among the sampled areas within the farm. Arthropod sampling occurred in each farm in a fortnightly basis with a sampling effort of 48 hs per farm per trap from March/2012 to February/2013. In the natural vegetation areas, the traps were kept 200 m from the forest edge along the entire experiment at the same place. As the Malaise traps sampled the most active flyers we complemented our samples with direct sampling of insects over the plants in each habitat. This sample method was performed at the same day we installed the Malaise traps. Groups of four samplers established linear transects crossing the plots in different directions and all the cropped and non cropped plants were entirely and carefully inspect in order to collect the arthropods on plants and above the ground. The arthropods were collected using plastic pots or manual aspirators, depending on their mobility and behavior. In each area the plants were inspect during 120 min, totalling a sample effort of 240 min per habitat in each month.

The arthropods collected in the Malaise jar after 48 hs and insects collected directly at the plants were send to the laboratory for classification and differentiation in morphospecies. Arthropods were identified as predators or herbivores based on the literature or comparing them with a pre-established collection of insects at the Embrapa Genetic Resources and Biotechnology, Brasília, DF, Brazil. When there was a lack of publication data, no specimens for comparison in the collection of insects or when we no identification was possible, the classification was made based on the external morphology such as mouthpart morphology. The non identified species were excluded from our analysis to avoid misclassification into the functional groups of herbivorous and predaceous arthropods. Moreover, when we were not able to classify the identified species into a functional group the specimens were also excluded from the analysis.

Statistical analyses

Previous to statistical analysis, we first pooled the data of species abundance per habitat from the fortnightly samples and transformed it into monthly abundance of predator and herbivore species by summing the specimen abundances. In a previous analysis we identified that the community composition of herbivore and predator arthropods were quite different between arthropod sampling methods used (Appendices, Figure A1). This indicates that the differences between methods could be complementary to assess the differences in artrhopods communities better than each method separatedly. Therefore, we also pooled the abundance data from the Malaise traps with the data from the direct sampling of arthropods over the plants. As there were many rare species of predators and herbivores, only for the analysis of abundance of predators and herbivores we excluded all species with fewer than 12 individuals collected along the year (*i.e.* less than one individual collected per month). Adopting this procedure, we avoided many unexplained residual in our analysis of abundance data. Moreover, removing these 264 species of predators and 210 species of herbivores from our samples reduced the number of predator and herbivore species analyzed in 66.08% and 50.12%, respectively. However, even excluding these species we kept up to 95% of the total abundance of individuals collected through the year in both groups.

The species richness of predators and herbivores were initially compared among habitats by rarefaction curves to estimate the species richness of each group with a different number of individuals collected in each habitat (Krebs, 1999). We also used the Rényi profile to compare the diversity of predators and herbivores in each habitat, separately. The Rényi diversity profile is an easy diversity ordering technique that ranks the sites from lowest to higher diversity of species and might complement the interpretation of rarefaction curves (Ricotta et al. 2002, Ricotta, 2003).

Hierarchical clustering analysis was used to show how predator and herbivore communities on different habitats clustered through the unweighted pair-group average (UPGAM) method calculated using the Jaccard index. A bootstrap of 100 randomizations was performed to test the consistency of the nodes in the dendrogram (Hammer et al. 2001). The cophenetic correlation coefficient was used to test the goodness of fit of the dendrogram in preserving the pairwise distances between the original unmodeled data points (Hammer et al. 2001).

The differences in the abundance of predators and herbivores in different habitats were evaluated by fitting a generalized linear mixed-effect model (GLMM), separately for each functional group. The predator or herbivore abundance was used as response variables, habitats as explanatory variables and date of sampling was used as random factor in this first analysis. We assessed the significance of variables included in the model using a F-test. When any parameter affected the explanatory variables it was removed from the original model and a new model was fitted and compared with the full model by a F-test. If no differences between models were achieved we accepted the simplest model. This procedure continued until reached a minimal adequate model (Crawley, 2007). The differences in abundance of predators and herbivores were compared among the habitats by contrast analysis (Crawley, 2007). Finally, an analysis for modeling the residues was performed. To investigate whether the abundance of predators or herbivores were affected by the habitat type, date of sampling and herbivore (in the case of predators) or predator (in the case of herbivores) abundance we again fitted a GLMM, but now using the farm identity as random factor. This analysis was performed as described above.

We also investigated whether the abundance of predators and herbivores on the main crop and on neighborhood crop were related to the number of vegetable species cropped through the year. To accomplish this, a regression analysis between the mean abundance per month of each group in each of these two habitats and the mean number of crops per farm per month was fitted separately for each group and for each habitat. We also run a regression analysis between the total abundance per species of predators and total abundance per species of herbivores, regardless of habitat type, to investigate whether the abundance of predators were conditioned to the abundance of herbivores. The diversity analyses were performed using the software PAST (Hammer et al., 2001) and all other analyses were performed using the software R (R Development Core Team, 2012).

Results

Diversity of predator and herbivore communities

A total of 79,947 arthropods were collected along the year. These arthropods were classified into 22 orders, 174 families and 1,695 morpho-species. Among these arthropods, 20,289 individuals divided into 12 orders and 55 families were classified as predators. The most abundant species of predators were *Condylostylus* spp. (Diptera: Dolichopodidae) (27.22% from the predators collected), *Toxomerus watsoni* (Curran,1930) (Diptera: Syrphidae) (5.77%), *Pseudodorus clavatus* (Fabricius, 1974) (Diptera: Syrphidae), *Diomus seminulus* (Mulsant, 1850) (Coleoptera: Coccinellidae) (4.02%) and *Scymnus* sp. (Coleoptera: Coccinellidae) (1.89%) (Table 1).

A total of 47,738 arthropods classified as herbivores was divided into nine orders and 53 families, and the most abundant species within this group were *Empoasca* sp. 1 (Hemiptera: Cicadellidae) (14.67%), *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae) (8.01%), Ulidiidae morphospecies 0226 (Diptera: Ulidiidae) (6.75%), *Empoasca* sp. 2 (Hemiptera: Cicadellidae) (6.53%) and *Euxesta* sp. (Diptera: Ulidiidae) (3.93%) (Table 1).

Table 1 – Total number of most abundant species of predatory and herbivorous arthropods in the main crops (Main), neighborhood crops surrounding the main crop (Neigh), fallow areas (Fall) and native forests (Forest) in organic farms cropping vegetables in the Brazilian Federal District, from March/2012 to February/2013.

	Abundance			
Taxon	**Main crop**	**Neighborhood crops**	**Fallow areas**	**Forest**
Predators				
Coleoptera				
Cantharidae				
Morphospecie 3.60	131	108	68	5
Coccinellidae				
Diomus seminulus	233	424	142	4
Scymnus sp.	108	133	89	28
Cycloneda sanguinea	47	94	40	1
Dermaptera				
Forficulidae				
Doru luteipes	75	79	48	0
Diptera				
Asilidae				
Ommatius sp.	15	65	49	0
Dolichopodidae				
Condylostylus spp.	1437	1788	1284	155
Syrphidae				
Toxomerus watsoni	759	960	269	29
Toxomerus politus	74	74	79	

Toxomerus dispar	85	90	30	3
Toxomerus lacrymosus	131	56	62	5
Pseudodorus clavatus				
Hemiptera				
Miridae				
Morphospecie 2.21	110	127	46	0
Hymenoptera				
Vespidae				
Polybia paulista	83	65	32	40
Polybia occidentalis	41	95	32	4
Neuroptera				
Chrysopidae				
Chrysoperla externa	27	73	69	1
Herbivores				
Coleoptera				
Chrysomelidae				
Diabrotica speciosa	1960	946	846	24
Morphospecies 3.368	238	370	172	15
Diptera				
Micropezidae				
Taeniaptera sp.	209	528	94	18
Tephritidae				
Morphospecies 0114	313	322	51	3
Morphospecies 092	469	355	51	0
Ulidiidae				
Morphospecies 0226	630	1702	758	47
Euxesta sp.	340	966	468	54
Morphospecies 0233	110	610	84	12
Hemiptera				
Cicadellidae				
Empoasca sp. 1	2358	2963	825	150
Empoasca sp. 2	927	1284	712	87
Ferrariana trivittata	283	145	600	0
Morphospecies 1.05	138	270	457	10
Morphospecies 1.130	167	498	604	108
Rhopalidae				
Arhyssus (cf.) sp.	646	651	216	2
Lepidoptera				
Crambidae				
Morphospecies 10.118	349	641	187	11

Comparing the species richness, it was observed that the fallow areas had more predator species than the other habitats, while the forests fewer species. The main crops and the neighborhood crops intermediate values of predator richness with very similar values between these two habitats (Figure 1a). For herbivores, there were no evident differences related to species richness among habitats (Figure 1b). However, the rarefaction curves for herbivores and predators in forests did not reach the plateu regarding species sampling. When comparing the diversity profiles, the predator and herbivore communities followed a similar pattern. There was a trend for a higher diversity of predators and herbivores in fallow areas and lower diversity in native forests. To the other habitats it was not possible to infer any differences in community diversity because the Rényi profile curves of predator and herbivore communities touch each other after alpha > 1 (Figure 2). Nevertheless, the community of predators and herbivores are dominated by a few abundant species. The 15 most abundant species shown in Table 1 for each group comprises 57.82% and 61.56% from the overall abundance of predator and herbivores in all habitats.

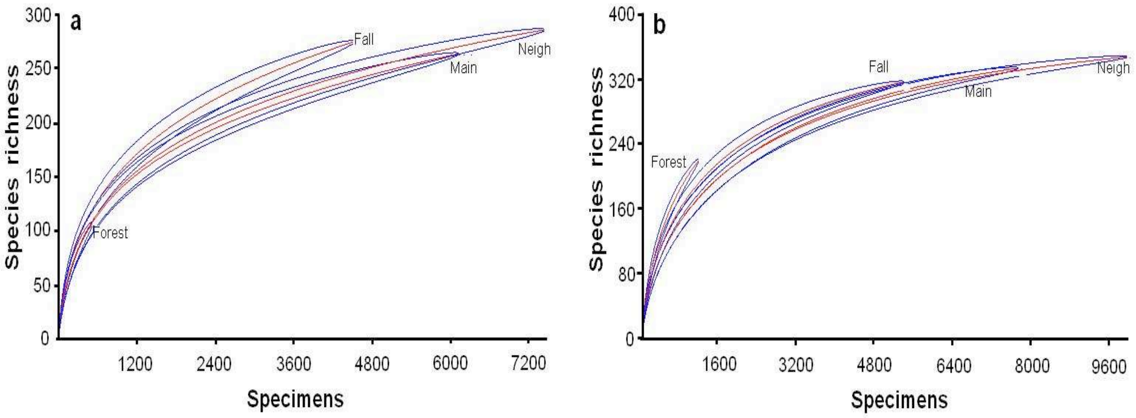

Figure 1 – Species richness estimated by rarefaction curves of predator (a) and herbivore (b) communities in the main crops (Main), neighborhood crops surrounding the main crop (Neigh), fallow areas (Fall) and native forests (Forest) in organic farms cropping vegetables in the Brazilian Federal District, from March/2012 to February/2013. Blue lines represent the confidence interval for rarefaction curves in each habitat. Note that 'y' axes are in different scales due to the differences in species richess of each functional group.

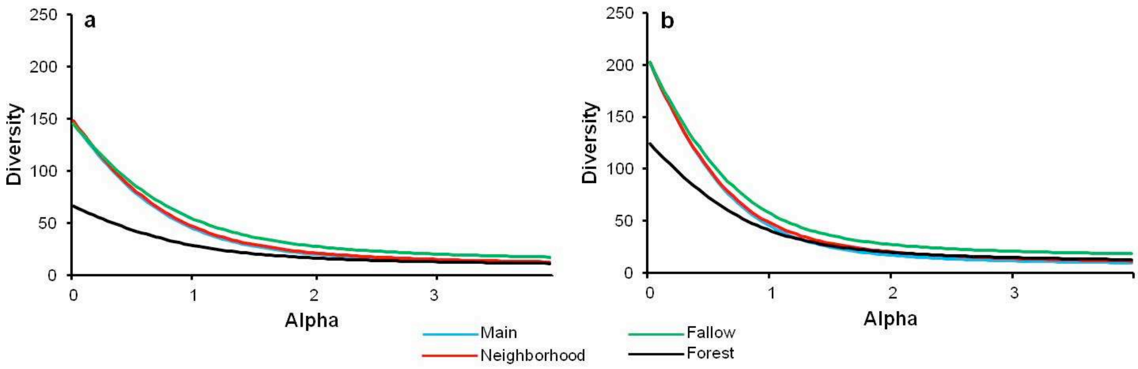

Figure 2 – Rényi diversity profile of predators (a) and herbivore (b) communities in the main crop (Main), neighborhood crops surrounding the main crop (Neighborhood), fallow areas (Fallow) and native forests (Forest) in organic farms cropping vegetables in the Brazilian Federal District, from March/2012 to February/2013.

Regarding the similarity of communities, the predator and herbivore communities followed the same pattern of sharing species among habitats. The main crop and neighborhood crops shared qualitatively (number of species) more species than the other habitats (Figure 3). Although the fallow areas shared less species with the main crop and neighborhood crops, it still presented very close similarity values with these habitats, indicating that at least 60% from the species collected occurred on these three habitat types (Figure 3). However, the natural areas are quite different in species composition when compared to the agricultural habitats (Figure 3), indicating that there was a shift in species composition if compared with the agricultural habitats.

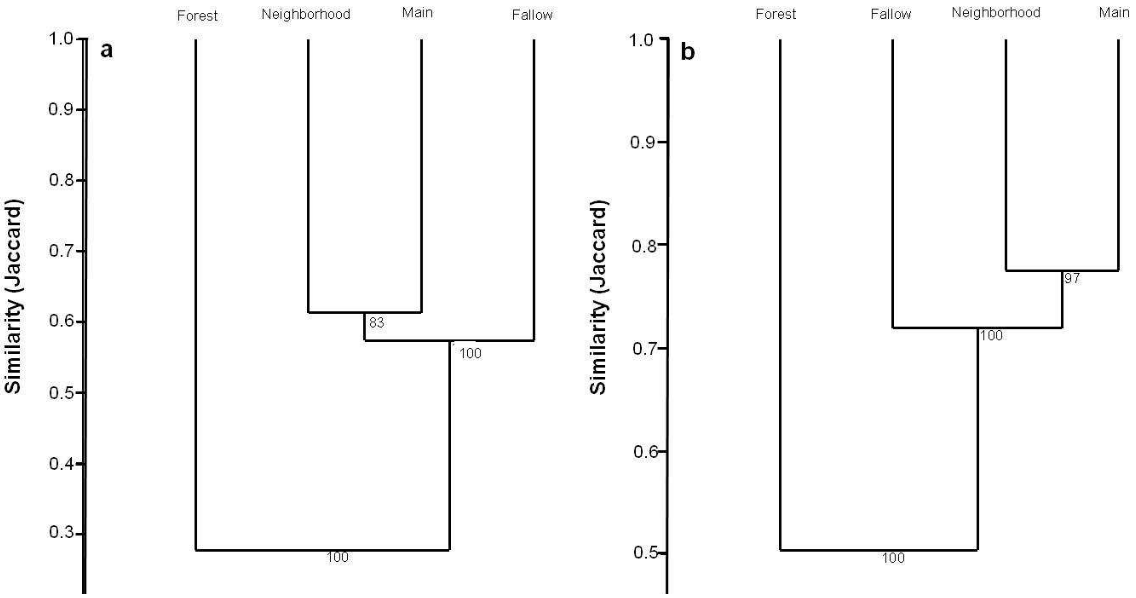

Figure 3 – Similarity in species identity and abundance of predator (a) and herbivore (b) communities in the main crop (Main), neighborhood crops surrounding the main crop (Neighborhood), fallow areas (Fallow) and native forests (Forest) in organic farms cropping vegetables in the Brazilian Federal District, from March/2012 to February/2013. Cophenetic correlation coefficient for predators = 0.997 and herbivores = 0.993. Number above each ramification indicates the consistence of nodes based on the bootstrap procedure with 100 randomizations. Note that 'y' axes are in different scales.

Abundance patterns of predator and herbivore communities

The overall abundance of predators was significantly affected by the habitat type (F = 21.18, 3 d.f., P < 0.0001). Predators were respectively more abundant in the neighborhood crops, main crop, fallow areas and forests (Figure 4). The habitat type also affected the overall abundance of herbivores (F = 13.82, d.f. = 3, P < 0.0001). Abundance of herbivores was higher in the main crop and neighborhood crops, which did not differ from each other, intermediate in the fallow areas and lower in forest areas (Figure 4).

When considering the temporal variability in the abundance of groups, we observed that different factors affected the abundance of predator and herbivores. Predator abundance was significantly affected by habitat type (F = 59.31, d.f. = 3, P < 0.0001), herbivore abundance (F = 285.03, d.f. = 1, P < 0.0001), date of the year (F = 4.49, d.f = 11, P < 0.0001), abundance of herbivores within each habitat (F = 4.01, d.f. = 3, P = 0.009), period of time in each habitat (F = 1.85, d.f. = 33, P = 0.0094) and by the herbivore abundance along the time (F = 4.48, d.f. = 11, P < 0.0001), but not by the interaction between date of the year, herbivore abundance and habitat type (F = 0.92, d.f. = 33, P = 0.5932). Herbivore abundance was significantly affected by the habitat type (F = 40.18, d.f. = 3, P < 0.0001), the abundance of predators (F = 215.38, d.f. = 1, P < 0.0001), the abundance of predators within each habitat (F = 3.94, d.f. = 3, P = 0.0112), the period of time in each habitat (F = 1.61, d.f. = 33, P = 0,042), the abundance of predators along the time (F = 2.02, d.f. = 11, P = 0.037) and by the interaction of predators within each habitat type along the year (F = 1.59, d.f. = 33, P = 0.04). No effect of the time period *per se* in the abundance of herbivores was detected (F = 1.41, d.f= 11, P = 0.1831).

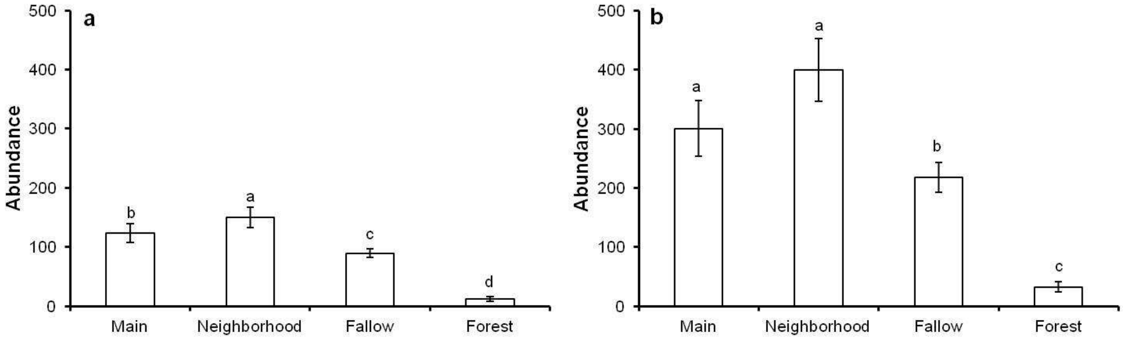

Figure 4 – Mean abundance (± SE) of predator (a) and herbivore (b) communities in the main crop (Main), neighborhood crops surrounding the main crop (Neighborhood), fallow areas (Fallow) and native forests (Forest) in organic farms cropping vegetables in the Brazilian Federal District, from March/2012 to February/2013. Means followed by the same letter did not differed significantly by the model contrast analysis (P < 0.05).

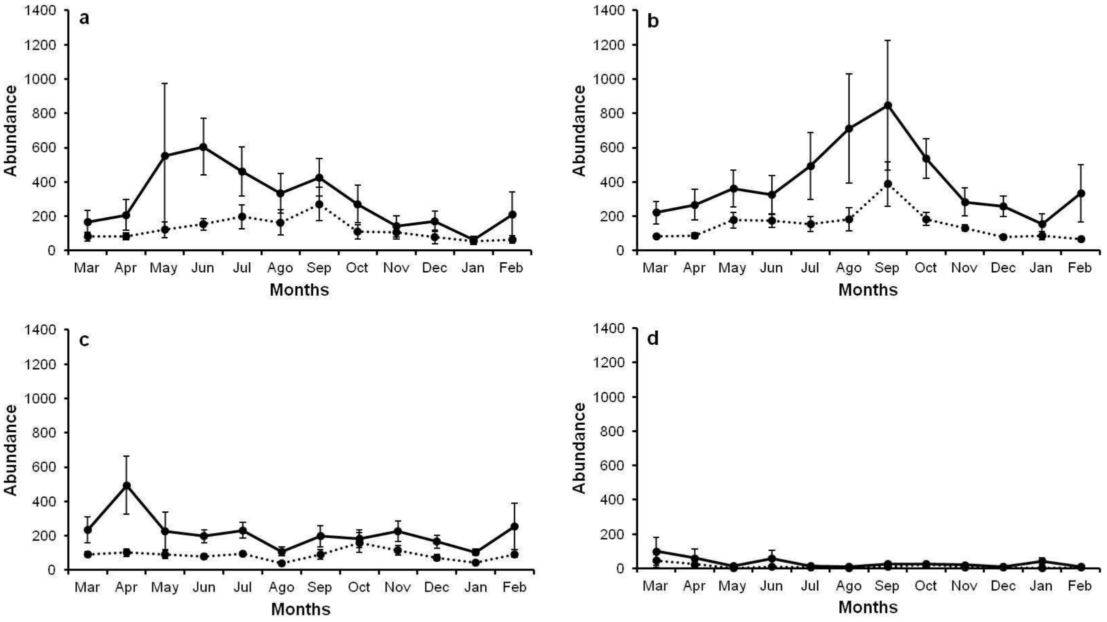

Figure 5 – Mean abundance (± SE) of predator (dashed lines) and herbivore (continuous lines) communities in the main crop (a), neighborhood crops surrounding the main crop (b), fallow areas (c) and native forests (d) in organic farms cropping vegetables in the Brazilian Federal District, from March/2012 to February/2013.

Thus, it was observed that the abundance of predators and herbivores were different among habitat types with higher abundances on the main crops and neighborhood crops (Figure 5). The abundance of herbivores presented two different peaks during the year in each of these two habitats, while the peaks of predator abundance where in September in both habitats (Figure 5ab). The fallow areas and forests presented more constant and close abundances of both groups along the year (Figure 5cd). But regardless of habitat type the abundance of predators and herbivores varied always together along the year (Figure 5). This relationship resulted in a numerical response to predators to herbivore abundance. The regression analysis showed that the abundance of predators was positively related to the abundance of herbivores, regardless of habitat type (Figure 6).

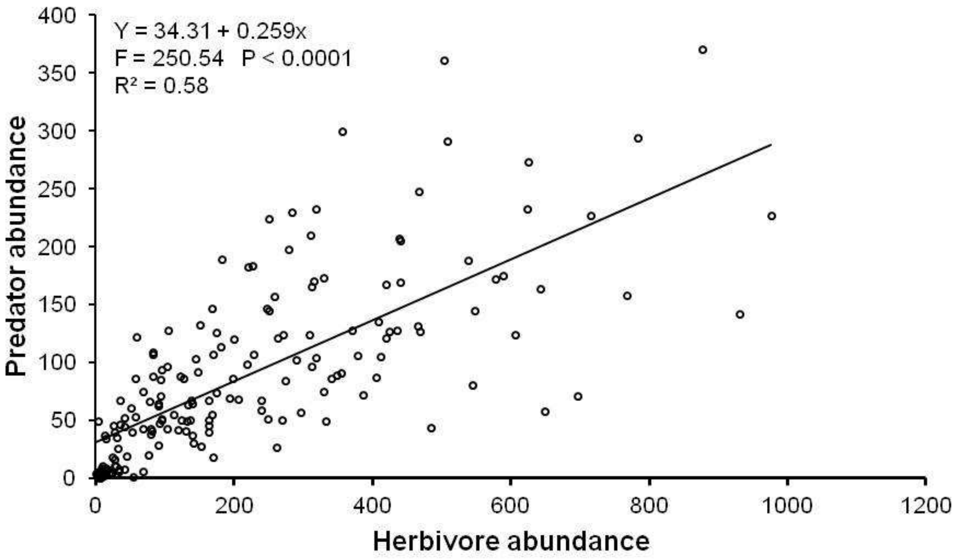

Figure 6 – Relationship between the total abundance of predators and herbivores in habitats within organic farms cropping vegetables in the Brazilian Federal District.

Discussion

The most abundant species of predators and herbivores in all habitat types were classified as generalist insects, because they can feed on several food sources. Predators such as the dipterous *Condylostylus* spp. and hoverfly species (*T. watsoni*, *T. politus*, *T. dispar*, *T. lacrymosus* and *P. clavatus*) are active flyers which can feed on soft-bodied herbivorous insects (e.g. aphids and whiteflies), and also use nectar and pollen from non-crop plants as food (Gerling et al., 2001, van Rijn et al., 2013). Other predators such as Chrysopidae and Cocccinellidae are also high mobile organisms because their prey (eg.: aphids or insect eggs) are ephemeral resources aggregated in patches within habitats; they can also use plant-provided food (Evans, 2003). The Asilidae and Vespidae species are known to chase and capture active preys such as lepidopterans and coleopterans or feed upon their larvae (Richter, 2000; Castelo and Lazzari, 2004).

Regarding herbivore abundance, the species collected can feed on several hosts. For example, *D. speciosa* is an important insect pest of beans, tomato, soybean and corn (Walsh, 2003). *Euxesta* sp. is found on maize crops, orchards and natural areas (Goyal et al., 2012). In our samples, the hemipteran *Arhyssus* sp. and the leafhoppers were found feeding on several species of weeds, grasses and on the Mexican sunflower and in different habitats. Generalist insects feed upon many prey/hosts in order to exploit different sources of nutrients obtained by a mixed diet (Bernays, 2001). These species need to have great mobility for finding suitable habitats with different resources and conditions, thus tending to constantly move between habitat types in the landscape in response to the availability of prey or hosts (Josen and Fahrig, 1997). Due to this, populations of predators and herbivores most likely move among habitats in a patch-dynamic approach or colonizing habitats in a mass-effect (Leibold et al., 2004).

The dispersal abilities of organisms are known to affect the local diversity of communities in time and space (Cadotte et al., 2006). In our study, we demonstrated that fallow areas contained the higher richness and diversity of predator species when compared with the main crop and neighborhood crops and native forest which presented a gradient with lower values. No evidence for differences in species richness of herbivores was achieved, but the diversity profiles in different habitat types showed exactly the same pattern observed for predators. Dispersion could be an important factor shaping community diversity of predators and herbivores, but other local factors related to habitat characteristics could have affected the species of predators and herbivores in each habitat.

This hypothesis was confirmed when we evaluated the similarity in community composition of predators and herbivores. There was a high similarity in species composition between the main crop and neighborhood crops with very small differences between predators and herbivores. Fallow areas also shared most species with the main crop and neighborhood crops, but with some exclusive species. The main crop and neighborhood crops are very similar habitats, providing similar resources and conditions for both groups, favoring that a similar pool of species were present on these habitats (Pandit et al., 2009). Fallow areas contained many weed species that also occurred in the cropped habitats that could be used as food sources by predators and herbivores (Bàrberi, 2002). Thus, the habitat composition of fallow areas shared some characteristics with the cropped habitats. But these areas are less subjected to disturbing factors such as total removing of weeds, harvesting, and crop species turnover. Some species can move to cropped habitats, but others apparently did not tolerate a frequent disturbing factor and remained in the fallow area. Therefore, agricultural habitats might provide species for each other in a source-sink dynamics due to their similarity in the resources therein (Mouquet and Loreau, 2003), with fallow areas presenting a key role on this dynamic.

The community composition in forests was quite different from agricultural habitats with many exclusive species. However, our sampling methodology did not assess a significative part of functional group communities in forests (no saturation of rarefaction curves), even when the data from different sampling methodologies were analyzed together. There are two main possibilities to explain this result. First, our sample methodologies were not sufficient to assess a representative part of arthropod communities because probably there is a great diversity of arthropods in such habitat. Second, the habitat structure and diversity are quite different from agricultural habitats and then arthropods could move at different stratas in the habitat affecting the samplying efficience. Independently of these possibilities, any inference considering our actual data about arthropod conservation in forests, should lead to nononclusive hypothesis, such as the limited value of forests for predator arthropods conservation. Moreover, studies dealing with such habitat types should also consider a more intensive sampling effort in native forests. On the other and, the possibility that forests within the farms could serve for conservation purposes of arthropod species from the Cerrado, which is a hotspot of biodiversity (Myers et al., 2000), and also to maintain other ecosystem services (Melo et al., 2013) is still unclear.

The overall abundance of predators and herbivores were also affected by habitat type. Predators were more abundant in the main crops and neighborhood crops. Herbivores also had higher abundance on neighborhood crops and did not differ from the main crop. Their abundance on neighborhood crops was very similar to the abundance on the main crop and fallow areas and lowest in forests. Despite the limitations of our sample methodologies, it is reasonable to assum that forests are highly diverse habitats, more complex in terms of odor sources and physical and visual barriers that could difficult predators finding their prey and herbivores finding their hosts (Wäschke et al., 2014). Fallow areas are an intermediate situation between cropping areas and forests. It remained unmanaged during all the experimental time which reduced the disturbing levels in this habitat. Plant-provided food from non-crop plants in this habitat can be used by different predators such as hoverflies and coccinellids and their survivorship are benefitted even when no prey is available (Amaral et al., 2013). At the same time, some of these plants can be used as hosts by herbivores. Thus, a higher diversity of resources could have favored the coexistence of more species of predators and herbivores with lower abundance than in other habitats, most likely due to niche partioning (Letourneau et al., 2011). In general, crops are more suitable habitats for herbivores because hosts are more abundant, homogeneous and predictable in the landscape and herbivores can achieve higher reproduction rates (Root, 1973; Altieri, 1999). Such habitats can also facilitate the movement of specimens between plants due to a lower diversity of plants (Wäschke et al., 2014). Generalist predators depend on the availability of their prey, and possibly such habitats also represent an immediate source of different preys during the crop cycles. Therefore, we can infer that the abundance of predators and herbivores were affected by different factors in each habitat.

This becomes more evident when analyzing the temporal variation in the abundance of predators and herbivores in each habitat. Besides the predator abundance is affected by the habitat type, we also found a significant interaction between habitat type and time. In fallow areas, the abundance of predators was more constant along the year, indicating that less disturbed and more diverse habitats might serve as source of individuals to cropped habitats during the entire year (Chaplin-Kramer and Kremen, 2012). We also found a significant interaction between the abundance of herbivores and date and an interaction between the abundance of herbivores in each habitat on predator abundance. Such results were most likely affected by the variation in the abundance of herbivores in the cropped habitats, because we only observed peaks of abundance on these habitats. When crops are harvested, the species therein should move to other habitats, such as fallow areas or adjacent crops. Nevertheless, when a new crop is planted there is a high availability of resources for herbivores that usually arrive into a habitat before natural enemies (Mazzi and Dorn, 2012). After the increase in abundance of herbivores the predators moving from adjacent habitats (*e.g.* fallow areas) might colonize the new cropped habitat and increased their abundance due to the availability of preys therein (Harterreiten-Souza et al., 2014). Thus, the proximity between the main crop and neighborhood crops could explain the similar abundance of predators in the main crop and neighborhood crops.

The abundance of herbivores was significantly affected by the habitat type, predator abundance and the interactions between these factors. In the Cerrado biome there is a well known seasonality in the abundance of many insect orders due to climatic conditions along the year in natural areas (Silva et al., 2011). In fact, variation due to stochastic factors was also reported by Harterreiten-Souza et al. (2014) in agroecosystems. The authors found that predators are more sensitive to such stochastic factors than herbivores, because the constant availability of food (cropped species) can favor a high abundance of herbivores during the entire year. Although we have found a similar pattern in our study we suggest that the variation in the abundance of herbivores within habitat types along the year in organic farms is most likely related to the abundance of predators in each habitat during a given period of the year in cropped habitats.

Furthermore, abundance of predators tended to be more constant than the abundance of herbivores in each habitat through the year, suggesting that they can move better between habitats than herbivores does. The movement of species between habitats depends on the habitat connectivity (Fiedler et al., 2008). In the organic farms that we sampled, the habitats were surrounded by diversified windbreaks, agroforestry systems, unmanaged weeds and orchards which could have facilitated the dispersion of predators within the farm. For example, agroforestry systems usually have more species of natural enemies than vegetable crops but in lower abundance, resulting in a qualitative source of beneficial insects to colonize vegetable crops when herbivores arrive (Harterreiten-Souza et al., 2014). In our study, fallow areas might have played a similar role in providing mainly predators for a rapid colonization of habitats with herbivores and establishing a numerical response between their community abundances.

The landscape composition at the farm-level in organic crops with different habitat types can affect the local diversity and abundance of predators and herbivores in a source-sink dynamics. However, the colonization and establishment of species qualitatively and quantitatively depends on the local management of such habitats. Maintaining habitats with reduced levels of disturbing factors, such as fallow areas, and with non-cropped species that provides resources (alternative or not) and adequate conditions, played a key role in conserving important species of predators. These species could disperse through the landscape and increase their abundance in crops in response of the increase of prey abundance, thus contributing to biological control and reducing damages on crops. Adjacent and similar habitats nearby the main crop should also be maintained in order to retain beneficial insects that could disperse in response to the increasing abundance of their prey in the main crop. Unfortunately, our sampling methodology was not sufficient to understand the role of native forest in conserving herbivores and predators in a tropical region. Therefore, new efforts considering other more intensive sampling plans should be made, particularly in a hotspot of biodiversity such as the Cerrado biome. Finally, farmers should keep habitats with different characteristics in order to conserve beneficial insects to the agricultural environment and also native species from the Cerrado biome. Using this strategy it is possible to design landscapes that compatibilize the needs for food production with biodiversity conservation, with farmers playing a central role in recoinciling such interests.

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CHAPTER II

Habitat diversity and reduced disturbance favor conservation biological control of *Bemisia tabaci* in tomato crops

Abstract

The whitefly *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) is one of the main pests of tomatoes worldwide. Recent studies suggested that biological control play an important role in controlling whitefly populations. Probably, increasing plant and habitat diversity within the farm, reducing disturbance levels, such as insecticide spraying, can broaden the effects of biological control agents over *B. tabaci* populations. We aimed to understand how different farm management strategies and diversity affected the conservation of natural enemies and the mortality of *B. tabaci* in tomato crops. We conducted a manipulative experiment in 20 small farms (five conventional and 15 organic). Farms were divided in four categories (five farms per category) forming a gradient of increasing farm diversity and lowering the disturbance levels by pest management practices. The richness and diversity of natural enemies was benefited by increasing farm diversity and lowering the disturbance levels. However, population levels of adult whiteflies were similar among farms in the beginning of the study, indicating a similar potential of colonization in all farms. The overall mortality of *B. tabaci* nymphs was very low in the conventional farms, moderate levels in the intermediate farm categories and very high at farm with high plant diversity and very low disturbance levels. Except by the conventional farms, predation was identified as the key-mortality factors in all organic farms and nymph dislodgement was also an important mortality factor. However, the contribution of biological control to nymph mortality was increased across the gradient of farms, mainly predation. Thus, indicating that less intensive management practices can favor the overall contribution of biological control to *B. tabaci* management. We also observed that increasing predator abundance and richness increased nymph mortality and reduced the variability of whitefly control. Therefore, increasing farm diversity and reducing adverse situations to *B. tabaci* natural enemies can significantly contribute to whitefly control, mainly in organic tomato crops.

Key-words: Whitefly, pest management, organic farming, predators, agroecology

Introduction

Conservation biological control consists of managing habitats in the agricultural environment to conserve and to enhance local natural enemy populations with as goal reduce the negative effects of pests on crops (Eilenberg et al., 2001). To this end, increase plant diversity within and around the crop fields has a positive effect on the abundance of arthropod natural enemies and a negative effect on herbivore opulations (Andow, 1991; Letourneau et al., 2009, 2011). That is because in diversified habitats, natural enemies can use different types of resources as well as shelter, suitable microclimatic conditions, oviposition sites and plant-provided food (pollen and nectar), leading to higher pressure on the populations of herbivores (Letourneau et al., 2009). Other important task in conservation biological control is to reduce adverse factors that could affect natural enemy communities (Naranjo and Ellsworth, 2009). There are several studies reporting the negative effects of disturbing factors, such as broad-spectrum insecticides spraying, on natural enemies which should be avoided in order to benefit biological control (*e.g.* Crowder et al., 2010; Arnó and Gabarra, 2011; Bommarco et al., 2011; Zhou et al., 2014). Thus, to make habitat manipulation a functional practice (*i.e.* effective biological control), a better understanding of the interactions among herbivores, natural enemies and crop management at the farm level is needed (Landis et al., 2000; Fiedler et al., 2008; Wyckhuys et al., 2013).

Organic farming is a suitable system to study these interactions because it is usually based on the use of ecosystem services by conserving the local biodiversity of natural enemies in order to reduce pest problems (Zehnder et al., 2007). Organic farming systems favor local biodiversity of several taxa, which contribute to pest control when compared with conventional systems (Hole et al., 2005; Bengtsson et al. 2005). These benefits are most likely related to the reduction in the use of synthetic insecticides and inorganic fertilizers, to the management of non-crop habitats and to the diversity of crops in the farm (Gurr et al., 2003; Zhender et al., 2007). However, a positive effect on biological control is not always achieved by increasing biodiversity of natural enemies (Straub et al., 2008), and this relationship between biodiversity conservation and pest control is highly variable among studies (Bengtsson et al., 2005).

In some situations key-species are responsible to the control of a target pest (*e.g.* Ives et al., 2005; Straub and Snyder, 2006). Therefore, no additive effect of natural enemy diversity in biological control is achieved. One can argue that in these situations the identity of species is more important than diversity and conservation efforts should focus on such species (Straub et al., 2008). The relationship between natural enemy diversity and biological control can be also negative or neutral. Negative effects can occur via intraguild predation or due to apparent competition, for example, and natural enemy species behavior and habitat structure may play a role in preventing such negative interactions (*e.g.* Venzon et al., 2001; Janssen et al., 2007). Neutral effects occur when natural enemies share similar niches and no gain in functionality is achieved by adding species to the natural enemy community (Ives et al., 2005; Straub et al., 2008). In other situations, there is an additive effect of natural enemy biodiversity on biological control due to, for example, complementarity in resource use, such as parasitoids attacking different phases of host development or during different periods across the time (*e.g.* Macfadyen et al., 2011). These contrasting relationships between natural enemy diversity and pest control indicate that there is a need to study case-specific situations for management purposes.

In vegetable crops, understanding the dynamics of natural enemies and pest control could be especially important, because they have a very fast crop cycle. For example, the cycle of tomato crops is approximately 180 days. In such ephemeral crops the efficiency of pest control by natural enemies could be directly related to the species pool available in the farm that could rapidily colonize a new habitat in response to an increase in herbivore populations (Tylianakis et al. 2005). Because of that, the provision of habitats such as agroforestry systems (Harterreiten-Souza et al., 2014), strips of weeds (Amaral et al., 2013), flowering plants (Togni et al., 2010) and non-cropped areas (Thies et al., 2003; Sujii et al., 2010) could serve as a source of these beneficial insects. Nevertheless, crop management at the plot level should also consider the availability of resources and conditions suitable for species colonization at the same time that adverse factors, such as broad-spectrum insecticide spraying, are mitigated (Naranjo and Ellsworth, 2009).

In the past years there is a growing body of studies demonstrating that natural enemies, mostly generalist predators, can be considered the most important mortality factor of the whitefly *Bemisia tabaci* biotype B Gennadius (Hemiptera: Aleyrodidae) (Naranjo and Ellsworth, 2005; Asiimwe et al., 2006; Karut and Naranjo, 2009; Naranjo and Ellsworth, 2009). *Bemisia tabaci* is a highly polyphagous herbivore that uses more than 500 plant species as hosts worldwide (Byrne and Bellows Jr., 1991; Oliveira et al.,

2001) and only in Brazil it causes an annual estimated loss of US$ 714 million (Oliveira et al., 2013). In fact, *B. tabaci* has numerous natural enemies including predators, parasitoids (Gerling et al., 2001; Arnó et al., 2010; Torres et al., 2014) and pathogens, such as fungi (Faria and Wraight, 2001), that can interfere with their population dynamic. The application of broad spectrum insecticides in an attempt to control high population levels of this pest can disrupt the natural biological control (Naranjo and Ellsworth, 2009). Naranjo and Ellsworth (2009) demonstrated that predators only played a role in the mortality of *B. tabaci* on cotton fields in USA when combined with selective insecticides, thereby reducing the pest populations into acceptable levels.

In tomato crops, *B. tabaci* is also considered a key pest (Kennedy, 2003). Tomato is one of the most important vegetables in the world. In Brazil, it is cropped in almost all regions under different management systems. However, the tomato cropped to be consumed *in natura* is produced almost esclusively by small growers with different farm designs and management strategies (IPD, 2010). Most common is the production of tomatoes in conventional systems, based on the constant use of synthetic insecticides and organic fertilizers. However, the organic tomato market is growing fast in Brazil (IPD, 2010). Despite the management system, the whitefly *B. tabaci* represents one of the most serious threats to the crop (Morales and Jones, 2004; Oliveira et al., 2013). In previous studies, we identified that tomato plants in the organic system are less suitable for whitefly development than in conventional fields, most likely because natural enemies are more abundant in the organic systems (Togni et al., 2009). Moreover, we observed that the local management of tomato crops by intercropping with a non-host species (*Coriandrum sativum*) and overhead sprinkler irrigation negatively affected *B. tabaci* host recognition and habitat suitability, respectively (Togni et al., 2010a; Togni et al., in prep.). These results suggest that the management of local factors on tomato crops can directly affect *B. tabaci* populations. Furthermore, the use of different strategies in organic crops such as intercropping and irrigation management in an integrative manner can broaden the effects over *B. tabaci* populations in organic tomato crops.

Therefore, farms presenting a higher diversity of habitats and plants and reduced levels of disturbance such as insecticide spraying, probably affect the conservation of *B. tabaci* natural enemies in tomato crops. The conservation of natural enemies can be directly related to their role as mortality factors of *B. tabaci* populations and thus vary with farm management. To test these assumptions we evaluated the abundance and diversity of *B. tabaci* natural enemies in four different categories of farms varying from conventional farms with a high input of chemical products and a very low diversity of habitats and plants to organic farms with a high diversity of habitats and plants and natural pest control. We also evaluated whether conservation of natural enemies was related to *B. tabaci* control and how it varied with farm management.

Material and methods

Location

This study was conducted in 20 small farms growing tomatoes in the Brazilian Federal District, Brazil. Small farmers in Brazil and in the Brazilian Federal District are the main producers of food for internal consumption (IPD, 2010). Especially organic farmers, have a high diversity of products per area planted in small farms (0.4 ha-12 ha). They are characterized by the employment of family labor in the farm, the use of different types of pest management strategies. Farms were located in the core of the Cerrado Biome, the Brazilian tropical savanna. The Cerrado biome is considered the main agricultural expansion area in the country, thereby consisting of a highly fragmented landscape (Ferreira et al., 2012). At the same time it is a hotspot of biodiversity (Myers et al., 2000).The climate of the region is Cwa semi-humid type, according to the Köppen-Geiger classification. Mean temperatures range from 22 °C to 27 °C, the average rainfall is 1,200 mm per year and the altitude ranges from 1,000 masl to 1,200 masl (Klink and Machado, 2005). There are two well-defined seasons throughout the year. The wet season is usually from November to March, whereas the dry season occurs from May to September. The experiments were conducted during the dry season in 2013 (May-September), a period when the average monthly temperatures vary from 15 °C to 30 °C, the relative humidity can fall below 15%, and the rainfall is < 100 mm. We selected this season for sampling because we could measure whitefly nymph dislodgment from plants due to overhead sprinkler irrigation without rainfall influence. No rainfall was observed in the sampled areas during the experiment.

Farm characterization

Before starting the experiment, we characterized the small farms and divided them in four categories according the following variables: a) Management system – conventional or organic (only certified farms); b) Use of windbreaks – when farmers used windbreaks we also evaluated the main species used in windbreaks and their disposal in the farm (*e.g.* in the vicinity or not from the tomato crop or dividing the area into cropped and non-cropped areas); c) Use of agroforestry systems – when agroforestry systems were present its disposal and approximate age was assessed; d) Number of cropped species besides tomatoes during the experiment; e) Number of crops planted around the tomato crop (*i.e* vicinity crops); f) Diversity of crops within the tomato plants plot – assessed by counting the number of crop species in polyculture with the tomato crop, if any; g) Weed management – in organic management systems we evaluated the weed management within and and surrounding the tomato crop (*e.g.* use of strips of weeds, and selective or complete weeding removal). In the conventional management systems the weeds were always totally removed by using herbicides; h) Irrigation management – drip, sprinkler and micro-sprinkler; i) Soil cover – exposed, plastic mulch, living ground cover, mulching with weeds and other plants; j) Fertilization – inorganic or if organic we also evaluated the type of fertilizers used (*e.g.* EM-bokashi, manure, natural termophosphate, green manure); k) Strategy used to pest control – varied from synthetic insecticides to natural pest control with no direct interference. When farmers used some product we also evaluated the identity from the most frequent products and classified them as synthetic, mixtures and biological; l) frequency of pest control – in farms with non-natural methods of pest control we evaluated the frequency of application of the main products used.

These variables were measured because we assumed that a higher diversity of plant species with different uses in the farm could affect the provision of resources and conditions for herbivore and natural enemy communities and could consequently affect the biological control and other mortality factors. At the same time, the characteristics about farm design and diversity of corpped and non-cropped areas represented the diversity of habitats in each farm. The habitat diversity was supposed to affect natural enemy conservation and biological control, similarly to plant diversity, but in a broader scale. Pest control strategies and soil management in the tomato crop were evaluated because these variables could affect the frequency of disturbance of insect communities (*e.g.* knockdown with insecticides and heat stroke due to soil exposure).

Based on the variables described above, farms were classified in four categories according to the habitat and plant diversity and disturbance level (five farms per category), as follows: Category I: conventional farms, high levels of disturbance and very low plant and habitat diversity; Category II: organice farms, moderate levels of disturbance and low plant and habitat diversity; Category III: organic farms, low levels of disturbance and moderate plant and habitat diversity; Category IV: organic farms, very low levels of disturbance and high plant and habitat diversity. All variables used to separate the farms into categories and the characteristics of each farm are described in the Appendices (Table A1). Sampling in all farms started when tomato plants where in between second and third week after transplanting, because tomato plants are more susceptible to whitefly infestation in this period (Giordano et al., 2005; Oliveira et al., 2013).

Insect sampling

Yellow sticky traps (15 cm x 25 cm) were used to evaluate adult whitefly densities and the abundance and richness of whitefly natural enemies in the same farms as described above. Five traps were placed per farm, spaced at least 5.0 m apart, and always at the height of the plant canopy between two tomato plants. The traps were maintained in field during five days which corresponded to the period between the first nymph counting on plants and the evaluation of nymph mortality factors, describe below. This period was chosen in order to evaluate the natural enemy species in the sampled area that could be responsible for nymph mortality, providing a more realistic data about species occurrence at a given time. After this period, the traps were removed and the number of species and the abundance of whitefly natural enemies were evaluated. We selected the *B. tabaci* natural enemies for sampling based on the lists of whitefly natural enemies provided by Gerling et al. (2001), Oliveira et al. (2003), Arnó et al. (2010) and also on the new records of whitefly natural enemies made by Torres et al. (2014) in Brazil. The number of adult whiteflies per trap was also counted. The whiteflies were identified as *B. tabaci* Biotype B based on testing 10 individuals per farm using RAPD markers as proposed by Lima et al. (2002).

Mortality factors experiment

In this experiment we aimed to know how mortality factors vary among the farm categories. Firstly, a stock rearing of whiteflies in a greenhouse (27 ± 4 °C) was established in order to obtain whitefly cohorts for the field experiment. For the rearing, *B. tabaci* Biotype B individuals were collected in fields near Brasília, Brazil (15°47′ S and 47°55′ W), and transferred to potted cabbage plants (40 days old) inside wooden framed cages (90 cm x 90 cm x 100 cm) covered with a fine mesh. Each cage contained six cabbage plants. After two months whitefly population was established in the greenhouse and no symptom of virus infection was observed on cabbage plants. It was necessary to ensure that none virus-infected whiteflies were taken to the field in order to avoid crop contamination on small grower farms. Groups of twenty adult whiteflies were randomly collected from the stock rearing and transferred to clip cages with a manual entomological aspirator. A sex ratio tending to females was assumed (Villas-Bôas et al., 2002), thus no sex differentiation was done. Clip cages were formed by a rectangular foam with a circular opening in the middle (50 mm diameter). A transparent plastic pot (30 ml in volume) was attached to the foam in the circular opening in the foam, using silicon glue. The upper side of the plastic pot was recovered with a fine mesh and the pot basis was cut. To close the cages, a rectangular transparent plastic with the same size of the rectangular foam was cut. The plastic rectangle was then fixed in the foam using two hairclips. Thus, the whiteflies were confined into the plastic pot attached to the foam and the rectangular plastic closed the system, producing an easy to open clip cage.

Clip cages were placed on twenty tomato plants randomly assigned in each farm. Each clip cage contained 20 whiteflies. All leaves were examined with a 15X magnifier to check if there were no whitefly eggs left by local populations before placing the clip cages. The clip cage side containing the plastic pot recovered with a fine mesh was positioned in the abaxial side of the tomato plant leaflet for adult feeding and the plastic rectangle in the adaxial side. Therefore, whiteflies could only feed and lay eggs in an area delimited by the plastic foam. Clip cages were left in the field during 48 h for adult oviposition. Using this method it was possible to standardize cohort age independently from the local population densities. Subsequently, the clip cages were removed and the leaf containing the leaflet with eggs was marked with a flagging tape. After 15-20 days, we counted the number of nymphs per plant in each farm using a 15X magnifier. This period correspond to the average time between egg development and the fourth instar. Moreover, whitefly nymphs are sessile and only first instar nymphs are mobile and only move a few centimeters after hatching (Byne and Bellows Jr., 1991). To avoid any bias in the mortality factor analyses we counted the number of live nymphs with the same age in each leaflet. Only two well trained observers counted the number of live nymphs. Five days later the infested leaflets were cut and taken to the laboratory to identify the mortality factors for each cohort. We identified the mortality factors only to the forth instar nymphs, which is the most susceptible phase of whitefly development (Naranjo & Ellsworth, 2005, 2009; Karut & Naranjo, 2009). Moreover, only during the fourth instar it is possible to identify parasitized individuals. The number of live individuals was also recorded in the leaflets taken to the laboratory.

Five different mortality factors were analyzed based in the descriptions of Naranjo & Ellsworth (2005). We considered that some individual was death by predation in two circunstances. First, when it was observed an empty transparent and wrinkled cutile the death was attributed to sucking predators. Second, when we found partially intact cadavers the death was attributed to chewing predators. Parasitized individuals presented a displacement of mycetomes or it was possible to identify the parasitoid pupae or larvae inside the host. Nymphs presenting evident color change or hyphal growth were considered dead by pathogens. Dislodgement was estimated adding the number of dead and live individuals in the second nymph sampling and subtracting this number from the total number of live nymphs in the first sampling. However, it was not possible to fully disentangle the effects of predation from dislodgement because it is reasonable to assume that chewing predator could eat the entire nymph. Therefore, we considered that individuals could have been removed by predation / dislodgement. Finally, all non evident cause of death, such as physiological death, was classified as unknown.

Statistical analyses

Species richness of natural enemies found on sticky traps was compared among the farm categories by rarefaction curves (Krebs, 1999). We used the Rényi function to compare the diversity profiles of natural enemy communities among the four farm categories (Tóthmérész, 1995). The Rényi diversity profile is an easy diversity ordering technique that ranks the sites from lowest to higher diversity of species and might complement the interpretation of rarefaction curves (Tóthmérész, 1995). To do this, Rényi diversity profile is based on the parameter ‘alpha’ which is a continuum of diversity measures related to each other by the Rényi function (see details in Ricotta et al., 2002, 2003). The Rényi profile curves plotted from the parameter alpha could be directly compared with each other and allow to make inferences concerning the diversity patterns among farm categories. However, when the diversity curves of two or more communities intersect, they could not be compared because in some instance at least one diversity estimator did not represent well the differences in the diversity patterns of these communities (Tóthmérész, 1995).

To evaluate whether the abundance of natural enemies was affected by farm category, we fitted a generalized linear mixed model (GLMM) using the maximum likelihood method (Legendre and Legendre, 2012). The abundance of natural enemies was used as response variable, the farm category as explanatory variable and farm identity as random factor. The model significance was assessed by a *F*-test. After that we compared the model with a null model using the same procedure described above. If significant differences from the null model were achieved we accepted the final model (Crawley, 2007).When differences in the abundance of predators were achieved a model contrast analysis was performed to assess the differences among farms (Crawley, 2007). Finally, a model residual analysis was performed. The abundance of whiteflies among farm categories was compared also using a GLMM, but number of adult whiteflies per trap was used as response variable, the farm category as explanatory variable and farm identity as random factor.

To analyze the nymph mortality factors it is necessary to consider that there is no obvious sequence of mortality causes because one cause of death can obscure the action of another (Naranjo and Ellsworth, 2005). Only the death by Dislodgment is considered to be an independent mortality factor because the removing of nymphs from the leafleats could not be obscured by any other contemporaneous mortality factor (Naranjo and Ellswoth, 2005).Therefore, the observed (or apparent) mortality rates of all other mortality factors were estimated as if that was the only mortality factor operating with no influence from other contemporaneous mortality factors. To avoid this methodological bias, we transformed the apparent mortality into marginal rates of mortality using the formulae $M_B = d_B/(1-d_A)$ proposed by Naranjo and Ellsworth (2005) after modification of the original formulae suggested by Buonaccorsi and Elkinton

(1990), where 'M_B' is the marginal rate of mortality of a given mortality factor, d_B is the apparent mortality of a given mortality factor and 'd_A' is the sum of all other relevant contemporaneous mortality factors. Subsequently, marginal rates were expressed in k-values using the formula $k = -\ln(1-M)$, where M is the marginal mortality rate of a given factor, for all subsequent analyses. These k-values where used because Varley and Gradwell (1960) discussed that this is a standardized value that is additive across mortality factors. After that it was evaluated whether the overall mortality rates (total $K = \Sigma\ k$) was affected by farm category fitting a GLMM, as described above. Key-mortality factors were quantitatively identified using the method of Podoler and Rogers (1975), which consists in regressing individual k-values on total K. The mortality factor with the largest regression coefficients (i.e. slope) was considered the key-mortality factor. After that, the k-value from the key mortality factor were subtracted from the total K and the remaining k-values were again regressed on the total K minus the key mortality factor. This procedure continued until remaining only two k-values from the remaining mortality factors. In this way it was possible examine the relative importance of all mortality factors that may be obscured by other mortality factors (Smith, 1973).

Death due to biological control (Pathogens + Parasitism + Predation) was identified as important mortality factors and then was analyzed separately. To accomplish this, we first evaluated the influence of farm category on the overall mortality due biological control by summing the individual k-values for death by predation, parasitism and pathogens producing a partial total K value. Then a GLMM was fitted using the overall mortality of nymphs due to biological control as response variable, farm category as explanatory variable and farm identity as random factor. We repeated this procedure for individual k-values of predation, parasitism and pathogens separately to evaluate how each mortality facto was affected by farm management.

Finally, in previous analysis predation was identified as the key-mortality factor in most farms and its importance varied among farm category. We then investigated whether the number of species and abundance of predators could affect the mortality rates and the variation of the mortality rates by predation in all farms. First, we excluded the parasitoid data from our analysis and fitted a linear model between the total number of predator species collected per farm and the average abundance of predators per farm with the average predation rates in each farm, separately. Subsequently we fitted another linear model between the total number of predator species collected per farm and the average abundance of predators per farm with the coefficient of variation (CV) of the predation rates per farm. All analyses were performed using the software R (R Development Core Team, 2012).

Results

Abundance and diversity of natural enemies

A total of 1,855 individuals known as whitefly natural enemies were collected and classified in seven orders, 15 families and 37 species (Table 1). The most abundant and frequent group of chewing predators were represented by coccinellids, in all farm categories. Regarding sucking predators the hemipterans were the most abundant and frequent group of nymph predators in the samples. The most abundant species in all farm categories were *Condylostylus* spp. (Diptera: Dolichopodidae), but the abundance and composition of natural enemy communities varied between the farm categories (Table 1). For example, *Macrolophus* sp. (Hemiptera: Miridae) was the second most abundant species in farm categories I and II and *Diomus seminulus* (Mulsant) (Coleoptera: Coccinellidae) the third most abundant species. Differently, in farm categories III and IV *D. seminulus* was the second and *Macrolophus* sp. the fourth most abundant species (Table 1).

Table 1 – Total abundance and proportion of *Bemisia tabaci* natural enemy species collected in different farm categories cropping tomatoes in the Brazilian Federal District, 2013. Farms were classified in four categories, forming a gradient of plant diversity within the farm and decreasing disturbance level of pest management practices from farm category I (low diversity and high disturbance levels) to farm category IV (high diversity and very low disturbance levels).

	Farm category				
Taxon	**I**	**II**	**III**	**IV**	**% from total**
Coleoptera					
Carabidae					
Carabidae sp. 1	0	0	0	2	0,11
Lebia sp.	0	0	1	16	0,92
Coccinellidae					
Delphastus sp.	4	2	12	25	2,32
Diomus seminulus	26	16	79	115	12,72
Eriopis connexa	2	6	6	2	0,86
Hyperaspis festiva	4	9	7	7	1,46

Nephaspis gemini	0	16	17	27	3,23
Nephaspis torresi	4	12	44	74	7,22
Olla v-nigrum	1	3	5	15	1,29
Coccidophilus sp.	0	0	6	5	0,59
Cycloneda sanguinea	2	0	6	4	0,65
Harmonia axyridis	2	2	2	4	0,54
Hippodamia convergens	0	2	2	4	0,43
Hyperaspis sp. 2	0	0	0	2	0,11
Scymnus sp. 1	0	0	0	5	0,27
Scymnus sp. 2	0	0	0	6	0,32
Serangium sp.	0	4	3	3	0,54
Stethorus minulatus	0	0	1	5	0,32
Nitidulidae					
Cybocephalus sp.	0	6	6	15	1,46
Diptera					
Dolichopodidae					
Condylostylus spp.	42	231	258	117	34,93
Syrphidae					
Allograpta sp.	2	2	14	17	1,89
Ocyptmaus sp.	0	6	19	31	3,02
Toxomerus sp.	3	1	2	2	0,43
Dermaptera					
Forficulidae					
Doru spp.	5	0	0	0	0,27
Hemiptera					
Anthocoridae					
Orius spp.	7	9	11	13	2,16
Berytidae					
Jalysus spinosus	0	2	3	7	0,65
Lygaeidae					
Geocoris spp.	2	1	6	4	0,70
Lygus sp.	7	9	14	17	2,53
Miridae					
Campylomma sp.	2	5	3	3	0,70
Campylotylus sp.	0	10	0	9	1,02
Macrolophus sp.	33	74	39	40	10,03
Spanagonicus sp.	0	0	0	2	0,11
Nabidae					
Nabidae sp.	0	2	0	0	0,11
Hymenoptera					
Aphenilidae					
Encarsia spp.	1	16	14	23	2,91
Neuroptera					
Chrysopidae					
Chrysoperla sp.	4	2	11	2	1,02

Hemerobiidae					
Hemerobiidae sp. 1	2	1	1	6	0,54
Spiders	5	14	8	3	1,62

When comparing the richness and diversity of natural enemy species among farm categories there was a gradient of diversity (Figure 1). Comparing the species accumulation curves for each farm category, the farms with higher plant diversity and low disturbance levels accumulated more species per sample than the other farms (Figure 1a). Nevertheless, this pattern was not much clear when observing only the rarefaction curves, because in farm category I, the rarefaction curves did not stabilized. However using the Renyi profile curves, it was observed that the communities of natural enemies in the category IV were more diverse than in all other farm categories, and categories III and II were slightly different from each other with a higher diversity in category III. However, we could not infer an overall difference related to community diversity when comparing category I with category II and III because the Rényi profile curve from farm category I touch the curves from category II and III at alpha > 1, indicating that these communities are not comparable by a diversity index (Figure 1b). Regarding the abundance of these natural enemies, there was a significant effect of farm diversity and disturbance level on individuals abundance (F= 6.33, 3 d.f., P= 0.005). Category I presented fewer individuals than category II and this latter category presented fewer individuals than category III and IV that did not differed from each other (Figure 2).

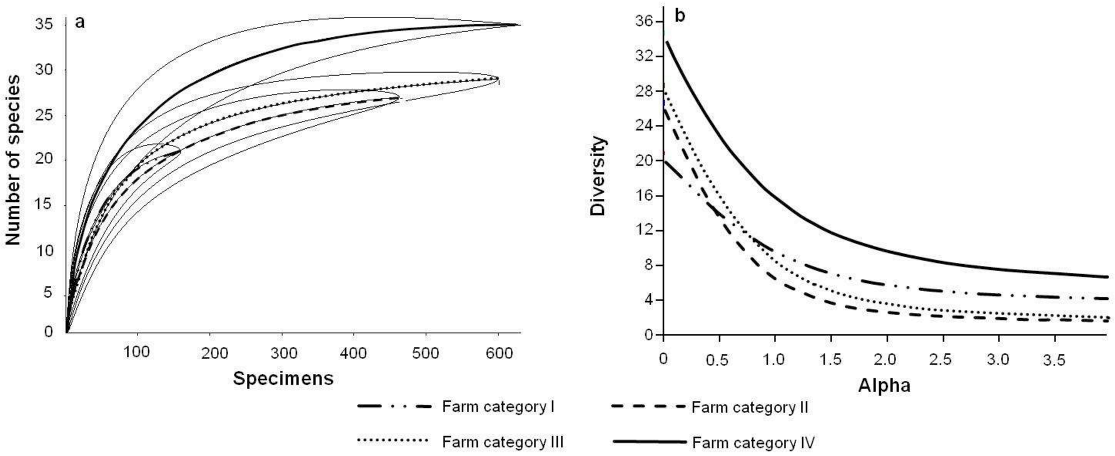

Figure 1 – Species richness estimated by rarefaction curves (a) and Rényi diversity profiles (b) of *Bemisia tabaci* natural enemies estimated collected in different farms cropping tomatoes in the Brazilian Federal District, 2013. Farms were classified in four categories forming a gradient of plant diversity within the farm and decreasing disturbance level of pest management practices from farm category I (low diversity and high disturbance levels) to farm category IV (high diversity and very low disturbance levels). Lines surrounding the rarefaction curves represent 95% confidence intervals for each farm category.

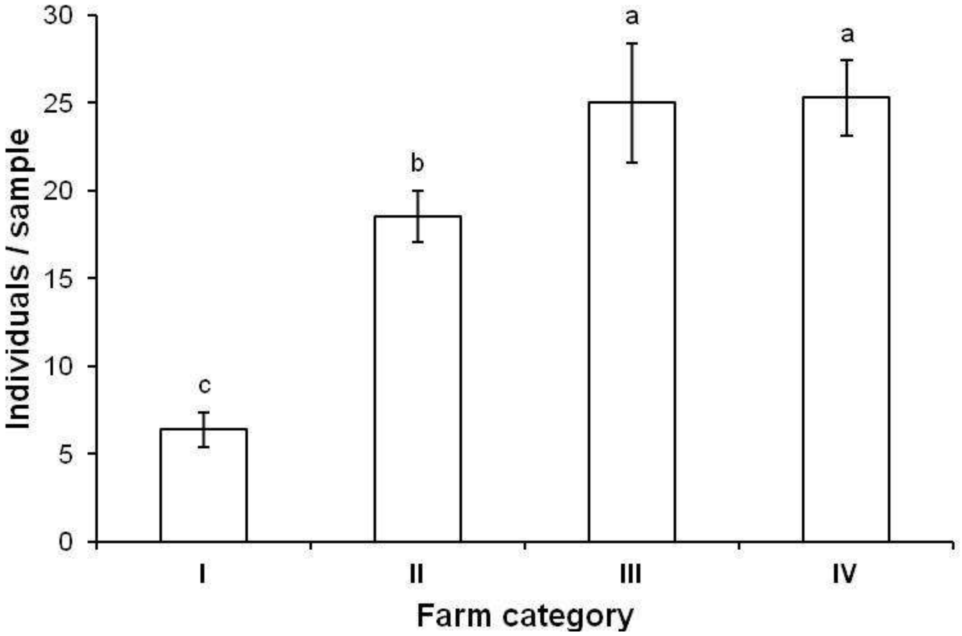

Figure 2 – Average abundance (± SE) of *Bemisia tabaci* natural enemies collected in farms cropping tomatoes in the Brazilian Federal District, 2013. Farms were classified in four categories forming a gradient of plant diversity within the farm and decreasing disturbance level of pest management practices from farm category I (low diversity and high disturbance levels) to farm category IV (high diversity and very low disturbance levels). Means followed by the same letter did not differ significantly ($P > 0.05$) by model contrast analysis.

Whitefly population levels

The population levels of adult whiteflies found on sticky traps did not differ significantly among the four farm categories (F = 1.65, d.f. = 3, P = 0.216) (Figure 3). However, when analyzing the descriptive statistics to perform the GLMM analysis it was observed that in farm category I there was a higher variation in the data, measured by the coefficient of variation (CV), and this variation declined constantly through farm categories until IV, which presented the lower levels of variation in the population levels of *B. tabaci* (Figure 3).

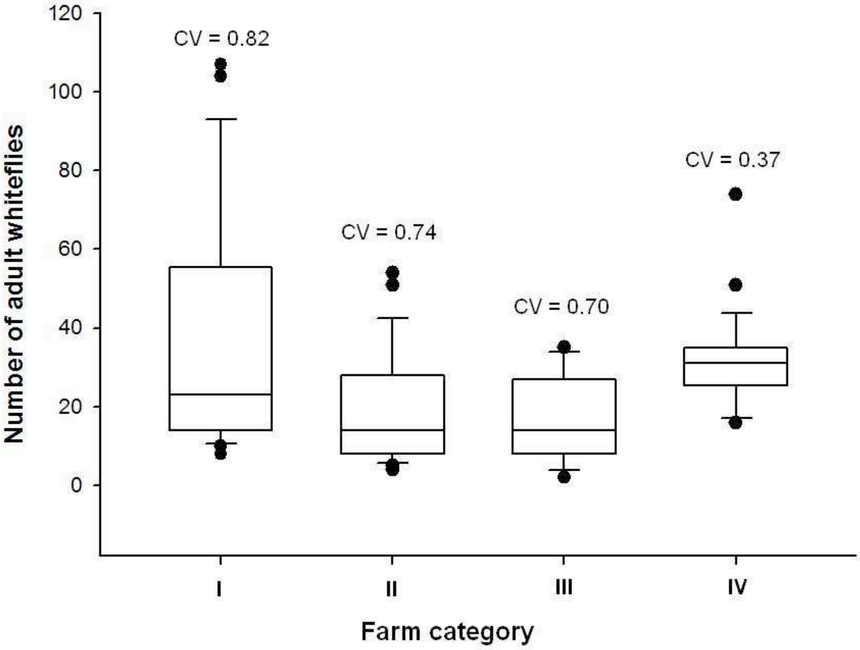

Figure 3 – Average number of *Bemisia tabaci* adults in different farms cropping tomatoes in the Brazilian Federal District, 2013. Farms were classified in four categories forming a gradient of plant diversity within the farm and decreasing disturbance level of pest management practices from farm category I (low diversity and high disturbance levels) to farm category IV (high diversity and very low disturbance levels). CV is the coefficient of variation of the abundance data within each farm category and point above and .

There was no relationship between the whitefly population levels and the number of natural enemy species found on sticky traps ($R^2 = 0.03$, F = 0.281, d.f. = 92, P = 0.597) and a very weak relationship with natural enemy abundance in each farm ($R^2 = 0.04$, F = 5.02, d.f. = 92, P = 0.027). Similarly, the number of natural enemy species

($R^2 = 0.05$, F = 0.95, d.f. = 17, P = 0.343) and abundance ($R^2 = -0.05$, F = 0.087, d.f. = 17, P = 0.771) were not related to CV of whitefly population levels in each farm.

Nymph mortality factors

The overall mortality of nymphs was significantly affected by farm category (F = 18.26, 3 d.f., P < 0.0001). Farms with higher diversity and less disturbance presented higher mortality rates (k-values) (Figure 4). From farm category I to categories II and III the overall mortality of nymphs increased in an order of about three times. In the same way the overall mortality of nymphs doubled from farm categories II and III to farm category IV (Figure 4). Thus, the overall mortality was increased across the gradient of farm diversity and disturbance (Figure 4).

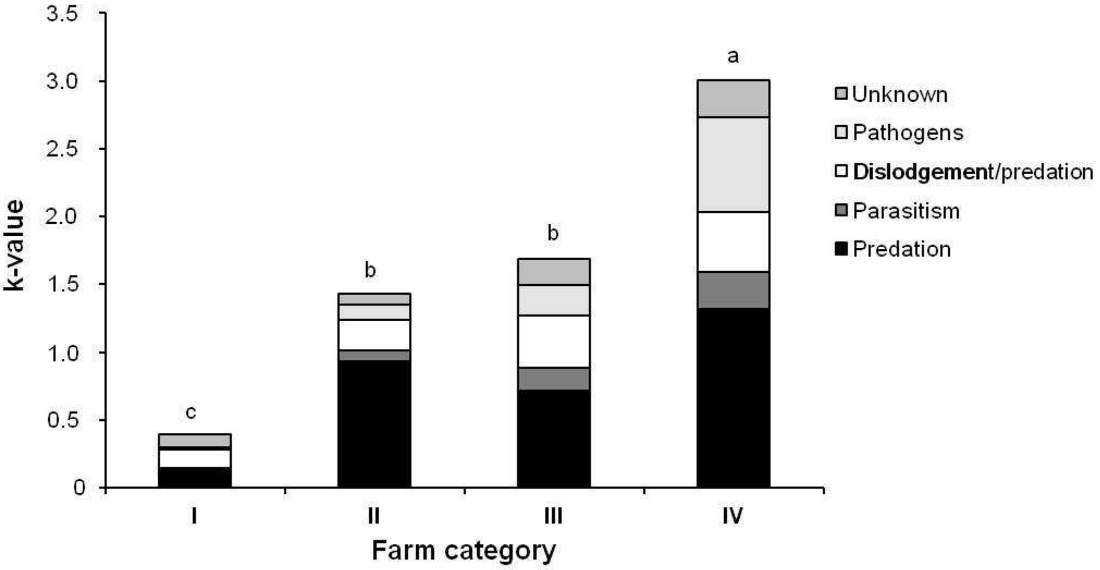

Figure 4 – Mean levels of mortality factors affecting the total mortality of *Bemisia tabaci* nymphs in farms cropping tomatoes in the Brazilian Federal District, 2013. Farms were classified in four categories forming a gradient of plant diversity within the farm and decreasing disturbance level of pest management practices from farm category I (low diversity and high disturbance levels) to farm category IV (high diversity and very low disturbance levels). Means followed by the same letter did not differ significantly (P > 0.05) on the overall mortality (Σ k-values) in each farm category by model contrast analysis.

Table 2 – Key-factor analysis of mortality factors affecting *Bemisia tabaci* nymphs in different farms cropping tomatoes in the Brazilian Federal District, 2013. Farms were classified in four categories forming a gradient of plant diversity within the farm and decreasing disturbance level of pest management practices from farm category I (low diversity and high disturbance levels) to farm category IV (high diversity and very low disturbance levels). Values represent the slope of regressions of k-values (partial mortality rates) of each mortality factor on total K (Σ k) for each farm category. At each step the largest slope were eliminated to evaluate the relative contribution of each mortality factor. Values in bold were considered key mortality factors in each farm category.

	Step			
Mortality factors	**1**	**2**	**3**	**4**
Farm category I				
Predation	0.152	0.701		
Parasitism	-	-	-	-
Pathogens	0.0004	0.013	0.042	0.491
Dislodgment/predation	**0.833**			
Unknown	0.038	0.261	0.892	
Farm categroy II				
Predation	**0.666**			
Parasitism	- 0.002	0.019	0.142	0.240
Pathogens	0.023	0.086	0.410	0.764
Dislodgment/predation	0.279	0.792		
Unknown	0.035	0.105	0.451	
Farm categroy III				
Predation	**0.417**			
Parasitism	0.027	0.061	0.093	0.234
Pathogens	0.054	0.135	0.341	
Dislodgment/predation	0.294	0.433		
Unknown	0.350	0.372	0.566	
Farm category IV				
Predation	**0.378**			
Parasitism	0.007	0.013	0.094	0.165
Pathogens	0.333	0.550		
Dislodgment/predation	0.141	0.225	0.496	
Unknown	0.142	0.212	0.411	-0.018

In farm category I, no parasitism was observed. Dislodgment;predation was identified as the key mortality factor, but predation also played a role. In the other farm categories, predation was always identified as the key mortality factor (Table 2). Although dislodgement;predation was a key mortality factor only in farm category I, in all other farm categories this factor had a prominent effect on nymph mortality. Pathogens only contributed significantly for nymph mortality in farm category IV (Table 2 and Figure 4). Unknown causes and parasitism were always a minor component of nymph mortality in all farms. Nevertheless, the contribution of each mortality factor to the overall mortality was increased across the gradient of farm diversity and disturbance, as observed by the apparent mortality rates (Table 2).

Role of biological control on nymph mortality

As mortality due to biological control comprised the major part of nymph mortality, at least in three from the four farm categories analyzed, we focused our further analysis on specific causes of death promoted by biological control. The overall mortality due to biological control represented 35%, 71.6%, 69.32% and 77.3% from the overall mortality due to all mortality factors together in farm categories I, II, III, and IV, respectively. The pooled data of mortality due to biological control (predation+parasitism+pathogens) was significantly affected by the gradient of farm diversity and disturbance (F = 23.99, 3 d.f., $P < 0.0001$). Higher mortality rates due to biological control were found in farm category IV, while farm category I presented the lower values, and categories II and III intermediate values (Figure 5). Decomposing the overall mortality into specific causes of death, parasitism was affected by farm diversity and disturbance (F = 8.74, d.f. = 3, P = 0.0012). However, these differences occurred because no parasitism was detected in conventional farms, thus significantly differing from other farm categories ($P < 0.05$). No differences were detected in the parasitism rates among organic farms (farm category II, III and IV) ($P > 0.05$). Death by pathogens was significantly affected by farm category (F = 11.35, 3 d.f., $P < 0.0001$). However, only in farms in the category IV the death by pathogens was significantly higher ($P < 0.05$ by model contrast analysis) than the other farms (no significantly differences among farm category I, II and III, $P > 0.05$). Nevertheless, death due to predation followed the same pattern of overall mortality rates with significantly differences among the farm categories (F = 14.193, 3 d.f., $P < 0.0001$). In the model contrast analysis predation rates presented higher values in farm category IV ($P < 0.05$), while farm category I presented the lower values ($P < 0.05$) and categories II and III intermediate values. Predation rates in farms in the category II and III did not differed significantly (P > 0.05). Thus, predation was the most important mortality factor that contributed to the observed differences in the overall mortality among farm categories.

Due to this, we investigated whether the abundance and number of species of predators could have affected the predation rates and the CV of predation rates in all farms. Farms with higher abundance and higher number of species of whitefly predators presented higher predation rates (Figure 6ab) and lower CV of predation rates (Figure 6cd).

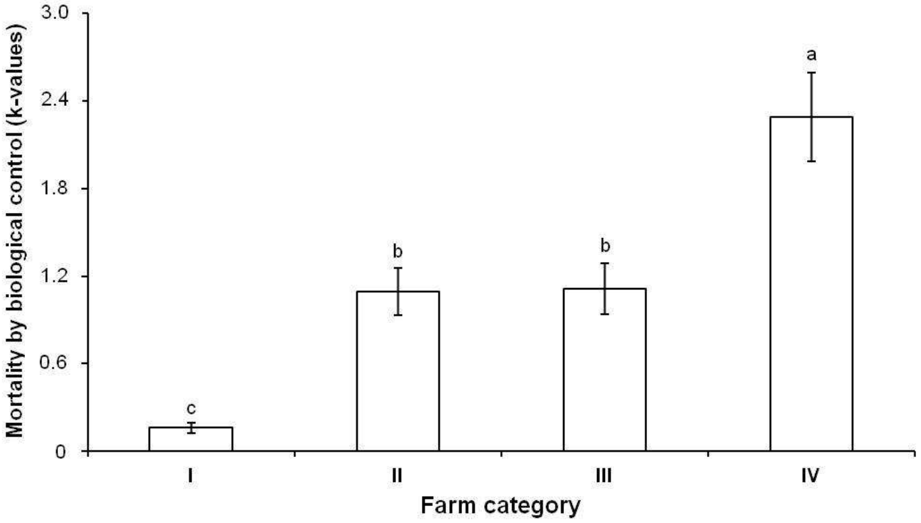

Figure 5 – Average mortality (±SE) of *Bemisia tabaci* nymphs by biological control (Predation + Parasitism + Pathogens) in small farms cropping tomatoes in the Brazilian Federal District, 2013. Farms were classified in four categories forming a gradient of plant diversity within the farm and decreasing disturbance level of pest management practices from farm category I (low diversity and high disturbance levels) to farm category IV (high diversity and very low disturbance levels). Means followed by the same letter did not differ significantly (P > 0.05) by model contrast analysis.

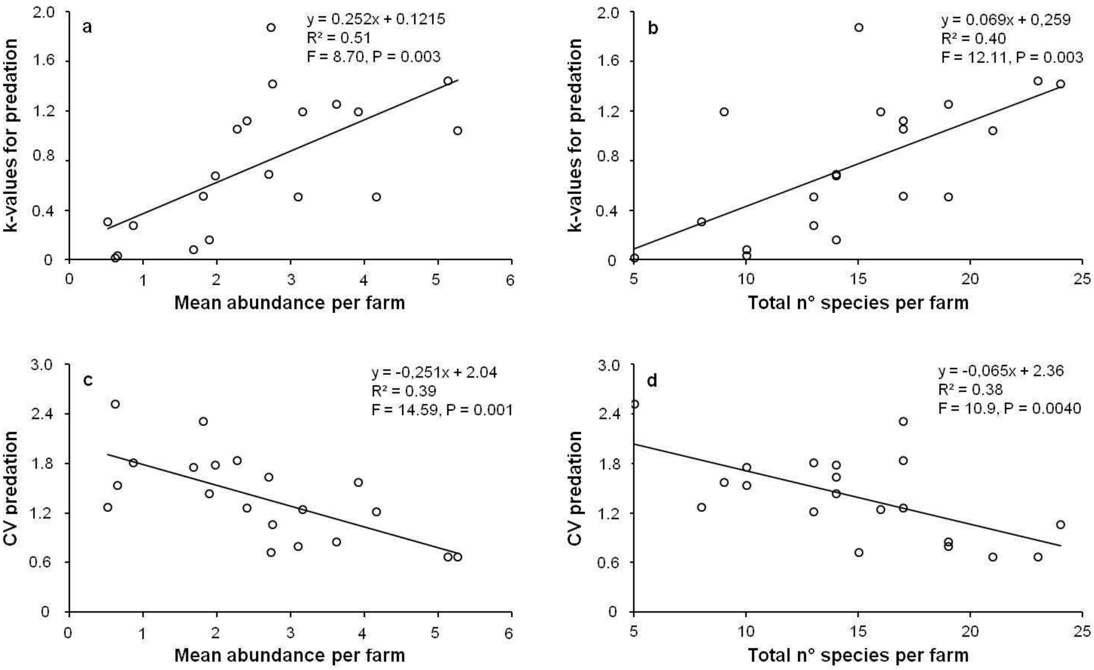

Figure 6 – Relationship between total mortality by predation (k-values) and mean abundance of predators per farm (a), total mortality by predation (k-values) and total number of predators per farm (b), coefficient of variation (CV) of the total mortality by predation (k-values) and mean abundance of predators per farm (c) and coefficient of variation (CV) of the total mortality by predation (k-values) and total number of predators per farm (d) in 20 small farms cropping tomatoes in the Brazilian Federal District.

Discussion

The diversity of natural enemies was positively affected by incread farm diversity and reduced disturbance levels by management practices of pest control such as insecticide spraying. Abundance of natural enemies also increased among farm categories, but at farms with low levels of disturbance and moderate plant diversity (category III), the abundance of natural enemies reached the maximum value and did not differ from farms with very low levels of disturbance and high plant diversity (category IV). Hence, increasing plant diversity and lowering disturbance levels can benefit the tomato agroecosystems by conserving more species of natural enemies (see Letourneau et al., 2011), mainly in organic farms (Letourneau and Goldstein, 2001; Togni et al., 2009). The mechanisms underlying these benefits for natural enemies could be related to the presence of more habitats such as weed strips, flowering plants, live ground covers and agroforestry systems in the more diverse farm categories. These farm traits can favor the coexistence of more species that use different types of resources in the same habitat (Bianchi et al., 2006). For example, agroforestry systems (perennial habitat) nearby horticultural crops serve as an important source of generalist predators that colonize the main vegetable crop (ephemeral habitat) and consequently favor conservation biological control (Harterreiten-Souza et al., 2014). Similarly, non-crop habitats provide a great amount of parasitoid (Macfadyen et al., 2011) and predator species (Thies et al., 2013). In some instance our sampling methodology could have omitted the presence of some predators, such as mites and other less mobile natural enemies. This could partially explain why the rarefaction curves for farm category I was not saturaded. However, in previous studies we used the same methodology and the most important species of whitefly natural enemies are usually sampled with a few non abundant species being excluded (Togni et al., 2009, 2010b).

Population levels of adult whiteflies were not affected by the characteristics of farm diversity and management, indicating similar potential for whitefly colonization of the tomato crops in all farms. Nevertheless, the population densities of adult whiteflies were highly variable in the less diverse and high disturbed farms than in the other farms. The colonization of new habitats by *B. tabaci* occurs when migrant flyers recognize specific light wave lengths from plants, which induce a photokinetic response (descendant flight) over long distances (Isaacs et al., 1999; Riis and Nachman, 2006). Following host or habitat acceptance, the whitefly population dynamic within a habitat is closely related to local factors, such as wind, temperature, spatial heterogeneity (Brewster et al., 1997), management practices (Togni el al., 2009) and semiochemical recognition (Bleeker et al., 2009; Togni et al., 2010b). Thus, it is possible that the management practices and farm design of each area have had an effect in the variation of the population density, producing different migration rates of adult whiteflies in each farm category. It resulted in different levels of variation among farm categories where the population densities of adult whiteflies on organic farms could be more predictable than the conventional farms. Natural enemies also did not affect the population levels of adult whiteflies. This might be because there are a few species known to be predators of adult whiteflies and in our sample we only collected two species (*Condylostylus* sp. and spiders) that potentially prey upon adult whiteflies (Gerling et al., 2001). This same result was previously observed by Togni et al. (2009) in organic and conventional tomato fields.

Alternatively, the similar abundance of adult whiteflies among farms indicates that different factors affected the recruitment of adults at farms with different management practices. In the conventional farms most likely artificial pest management practices were more important than biological control. But the extent that habitat and plant heterogeneity was increased and disturbance levels decreased, biological control could have played a major role in adult recruitment producing a similar result from that in conventional farms. Therefore, the ecosystem service of biological control probably substituted the need for artificial inputs such as insecticide spraying in some farms. Furthermore, if the ecosystem services compensate the need for artificial and intensive pest control it can be considered a more sutainable management practice of whitefly control and suitable for organic growers.

This hypothesis was confirmed when analyzing the nymph mortality in each farm category. The overall nymph's mortality was very low in the conventional farms, intermediate levels in farms with moderated levels of plant diversity and low disturbance levels (categories II and III) and very high at farms with high plant diversity and very low disturbance levels. Except for conventional farms, predation was the key mortality factor of nymphs in the field. Predation was also identified as a key mortality factor of third and fourth instar nymphs of *B. tabaci* in cotton fields in the USA (Naranjo and Ellsworth, 2005) and in Turkey (Karut and Naranjo, 2009) and played a significant role in cassava field in Uganda (Asiimwe et al., 2007). Moreover, in these studies, the authors observed that sucking predator species were the primary responsible for predation. In our study, chewing predator species were more common in our samples, because we have found many partially intact cadavers. Despite these differences, the ecosystem service of biological control was maintained independently from the community composition. Furthermore, we demonstrated that increasing habitat complexity and lowering disturbance levels can favor the predator community and consequently broaden their actuation on whitefly biological control in organic farms. On the other hand, releasing whiteflies from natural enemy control by the use of broad spectrum insecticides in conventional tomato crops, can favor whitefly population increase (Naranjo and Ellsworth, 2009).

Another important mortality factor identified was the dislodgment of nymphs in all farm categories. In conventional farms dislodgement, was identified as the key mortality factor, most likely due to the absence or very low impact of other biotic factors such as predation on nymph mortality. It is difficult to completely disentangle the effects of predation from dislodgment, and our inferences about the major role of predation in our samples are limited by this methodological issue. Nevertheless, the nymph dislodgement can be due to intrinsic or extrinsic factors such as when a newly formed nymph fail to reinsert its mouth parts into the plant tissue (Walker and Perring, 1994) or due abiotic conditions (*e.g.* wind speed, temperature, relative humidity) (Naranjo and Ellsworth, 2005), respectively . Other extrinsic factor that could be manipulated to broaden the effects of dislodgment is the irrigation management with overhead sprinkler irrigation. The water drops over tomato plants could dislodge nymphs and difficulty adult establishment in the crop as previously demonstrated in cotton (Castle et al., 1996) and in tomato fields (Togni et al., in prep.). Thus irrigation management can turn the crop as an inappropriate habitat for whiteflies by disturbing adults and dislodging nymphs. However, in our study only a few organic farms used this strategy.

Despite the importance of dislodgement, biological control was the most important factor of immature *B. tabaci* mortality. In general, the overall mortality due to biological control agents was significantly affected by farm diversity and management. However, each biological control agent (pathogens, parasitoids and predators) responded differently to farm categories. Parasitism was not observed in the conventional farms, probably due to broad-spectrum insecticides negative effects (Naranjo and Ellsworth, 2009). Although parasitism is the minor component of the mortality due to biological control agents, no differences in parasitism among organic farms was observed. Therefore, solely cropping tomatoes in the organic system was sufficient to maintain the ecosystem service of whitefly parasitism.

Although pathogens are well known biological control agents of adult and nymphs of *B. tabaci* (Faria and Wraight, 2001), they played a significant role in the nymph mortality only in the farms classified in the category IV (organic farms with very low disturbance and high plant diversity). On these farms, pathogens contributed to double the overall mortality due to biological control agents in comparison with other farm categories. This could have occurred due to a better microclimatic condition, availability of perennial habitats such as agroforestry systems and to less intensive soil management, where many species of pathogens can live (Klingen et al., 2002).

Therefore the pathogens had an additive effect to other biological control agents and contributed to an increase in the overall mortality of nymphs due to biological control.

Predation, as discussed above, played a major role on nymph mortality and it is the most important factor that contributed to nymph mortality due to biological control agents. Furthermore, increasing the abundance and richness of predators leads to higher predation rates of *B. tabaci* nymphs. This relationship of biodiversity of predators and herbivore suppression has gained more attention in a decade where biodiversity-friendly landscapes are emerging as important tools for ecosystem services conservation (Melo et al., 2013). As a general trend, increasing the vegetational diversity has a positive and direct effect on predators leading to more efficient pest suppression (Letourneau et al., 2009; Letourneau et al., 2011). However, these effects could be context dependent where in some cases key-species can control pests rather than multiple predator communities (Straub et al., 2008), while in other contexts predator or parasitoid diversity can positively impact biological control (Snyder et al., 2006; Macfadyen et al., 2011). Our study supports the hypothesis that biodiversity of whitefly natural enemies is directly related to pest control, probably due to complementarities in resource use of different species of predators. Other important result found here is that biodiversity and abundance of predators reduced the variability (CV) in whitefly mortality. Therefore, a more diverse and abundant community of whitefly predators resulted in a more reliable and constant control of whiteflies. Possibly, this could partially explain why the population of adult whiteflies in category IV has lower CV than the other farm categories. Similarly, Macfadyen et al. (2011) found that parasitoid richness resulted in lower variation in pest control across the time in organic farms, while this relationship was not achieved in conventional farms. Therefore, a higher richness and abundance of predators is also related to the reliability of whitefly control, mainly in organic crops.

In our study we have demonstrated that organic farms with a higher diversity of plants and habitats and low levels of disturbance by pest control strategies can directly benefit the diversity and abundance of natural enemies, leading to a more constant and reliable *B. tabaci* biological control on tomato crops. However, solely being organic has a limited impact on *B. tabaci* mortality. To broaden these positive effects it is necessary to maintain farms with different habitats, a high diversity of plants (cropped or not) and to use less intensive pest management practices. Generalist predators were the most important mortality factor of *B. tabaci* found in organic farms and their conservation in the farm increases the predation of nymphs and reduced the variability in pest control. Farmers would benefit from these results by designing their farms taking into account that an efficient and reliable ecosystem service of biological control of whiteflies depends on less intensive management practices and farm diversity in different levels. Furthermore, combining different strategies of management is imperative for the prevention of losses caused by this worldwide pest.

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CHAPTER III

Coriander benefits a generalist predator and can favor conservation biological control in tomato crops

Abstract

Manipulating plant diversity has been used to improve biological control for a long time. For example, intercropping tomato plants with coriander was demonstrated to attract several natural enemies of aphids, mostly coccinellids, but the mechanisms responsible by predator attraction was not fully understood. We aimed to investigate why coriander plants attract predatory coccinellids, using *Cycloneda sanguinea* as a model. We performed a series of controlled experiments with coriander plants in the vegetative and reproductive stages to exploit how *C. sanguinea* adults and larvae search for such plants. In a Y-tube olfactometer, *C. sanguinea* was attracted by coriander volatiles produced during the vegetative plant phase. The attraction to coriander volatiles was increased when offered aphid-infested tomato plants to *C. sanguinea*. Moreover, females also used coriander plants as oviposition sites, regardless of the plant age which could partially explain the attraction to coriander volatiles during the vegetative plant phase. Larvae born in coriander plants were able to find nearby aphid-infested tomato plants and contributed to aphid's biological control, mostly after three days. During the experiments we observed that *C. sanguinea* has some preference to use these plants as shelter when coriander is near blooming. That is because adults could feed on pollen and/or nectar from coriander flowers and their survivorship was positively affected even when no aphids where available, but with more prominent effect when aphids were also available. By the other side, females laid eggs only when fed with aphids and no significant effects in their reproduction were observed when supplementing their diets with coriander flowers. In conclusion, coriander plants are attractant to *C. sanguinea* because the plant at different development stages can benefit the predator adults and their offspring with implications on biological control.

Key-Words: *Cycloneda sanguinea*, Coccinellidae, agroecology, intercropping, plant volatiles

Introduction

Coccinellids are important biological control agents of aphids and other soft-bodied insect pests because larvae and adults are voracious predators (Obrycki et al., 2009; Michaud, 2012). Their main prey (aphids) is an ephemeral resource that occur aggregated in patches within a habitat (*e.g.* a crop) forcing coccinellids to move among habitats and find suitable patches for feeding and reproducing (Ferran and Dixon, 1993; Evans, 2003; Hodek and Evans, 2012). When searching for habitats, coccinellids can assess a wide array of sensory information. At some distance adults can use visual cues such as plant architecture and habitat shape (Nakamuta, 1984). Semiochemicals can also play a role in habitat/patch selection of coccinellids by signaling prey quality (Sarmento et al., 2007; Petterson et al, 2008; Hodek and Evans, 2012), non-prey food (Schaller and Nentwig, 2000, Ninkovic et al., 2001; Choate and Lundgren, 2013), competitors and intraguild predators (Sarmento et al., 2007), prey alarm pheromones (Al Abassi et al., 2000; Petterson et al. 2008; Cui et al., 2012), and partners (Petterson, 2012). Larvae can also use semiochemicals and identify tracks from other larvae to reduce foraging time for prey (Hemptinne et al., 2000). These sensory cues can help coccinellids to integrate information about the habitat and take a decision when searching for oviposition sites, shelter and prey in order to maximize the fitness of females and their offspring (Evans, 2003).

Generalist aphidophagous coccinellids have broad feeding habits and a life history associated with some plants and non-prey food (Giorgi et al., 2009; Lundgren, 2009; Magro et al., 2010). As they respond to different stimuli from the environment, provision of prey (essential and alternative), plant-provided food, shelter and oviposition sites via introduced plant can increase their role on aphid's biological control (Evans, 2003; Lundgren, 2009; Obrycki et al., 2009). For example, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) aggregated in patches within alfafa fields that contained dandelions, which increased the predation of aphids (Harmon et al., 2000). Similarly, *C. sepetempunctata*, *Adalia bipunctata* L., *Propylea quatuordecimpunctata* L. (all Coleoptera: Coccinellidae) also aggregated in lettuce plots interspersed with weeds (Sengonca et al., 2002). In both studies, the attraction of coccinellids to areas with higher botanical diversity was most likely because weeds can provide suitable conditions and resources such as pollen and nectar to coccinellids. Indeed, pollen and nectar can provide substantial amounts of energy and nutrients to coccinellids impacting their survival and reproduction (Lundgren, 2009). However, it is necessary to study case-specific interactions between coccinellids and non-prey food because these (positive or negative) effects can depend on the life history association between the organisms (*e.g.* Amaral et al., 2013).

One strategy that showed to be attractant to predatory coccinellids was the intercropping of tomato plants with coriander. Togni et al. (2009, 2010) registered a higher abundance of coccinellids in plots with tomato plants intercropped with coriander and Resende et al. (2010) noted the same pattern when kale was intercropped with coriander. These studies found that coccinellids were more attracted to flowering coriander plants, but higher abundance and diversity of coccinellids were observed in intercropped than in the monocultured plots even when coriander plants were in the vegetative stage. Furthermore, there is some evidence that coccinellids can feed on pollen and nectar from coriander plants (Medeiros et al., 2010), and use plants as oviposition sites and shelter (Lixa et al., 2010). The combined results of these experiments suggests that not only providing prey and non-prey food are involved in coccinellid attraction to habitats with coriander plants, and other factors such as the role of semiochemicals should be also considered.

The coccinellid *Cycloneda sanguinea* L. was one of the most abundant species in plots with tomato plants intercropped with coriander (Togni et al., 2010) and is one of the most common species in the Coccinellidae community in Brazil (*e.g.* Sujii et al., 2007; Martins et al., 2009; Harterreiten-Souza et al., 2012). It has generalist feeding habits preying on several aphid species, and it can feed on plant-provided food (Amaral et al., 2013). It is able to recognize odors from aphid-infested tomato plants and to discriminate between plants with a superior prey (an aphid) and an inferior prey (a mite) (Sarmento et al., 2007). Due to these characteristics, *C. sanguinea* is considered a promising biological control of aphids on tomato plants (Oliveira et al., 2005; Sarmento et al., 2007).

Here we investigated why coriander plants are attractive to *C. sanguinea* during different stages of plant development, because in field this predator is attracted to coriander plants in the vegetative and flowering phases. We first carried out a series of olfactometer experiments to test the role of odors from coriander plants in the vegetative stage in the predator foraging behavior. Subsequently, we performed a cage experiment to test whether plant phenology before flowering affect females choice for oviposition sites. As we observed eggs in coriander plants in the cage experiment it was investigated whether larvae born in coriander plants were able to find nearby aphid-infested tomato plants. Finally, we assessed the suitability of coriander flowers as alternative food for *C. sanguinea*. Understanding why coriander plants are attractive for this is species can contribute to a more effective habitat management in order to favor conservation biological control of aphids in field.

Materials and Methods

Plants and insects

Tomato plants, *Solanum lycopersicon* cv. "Santa Clara" (Solanaceae), and coriander plants, *Coriandrum sativum* cv. "Verdão" (Apiaceae), were sown in plastic pots (5 L) containing soil and a commercial plant substrate (Bioplant®) (proportion 3:1) in a greenhouse. Tomato plants (one per pot) and coriander plants (10 seeds per pot) were kept inside a wooden-framed cage (0.7 x 0.7 x 0.68 m) covered with a fine-mesh (90 µm) to avoid herbivore contamination.

The aphid *M. persicae*, one of the most abundant species in commercial fields in Brazil, and the predator *C. sanguinea* were collected on non-crop plants and on horticultural crops in the municipality of Piranga, Minas Gerais, Brazil (20º45′4′′ S and 43º18′10′′ W) in the agronomy experimental field and in an orchard at the Federal University of Viçosa, Minas Gerais, Brazil (20°45′14′′ S and 42°52′54′′ W). Insects were collected fortnightly from September 2011 until February 2012. The collected aphids were transferred to cabbage plants (50 days old) kept in plastic pots (5 L) inside wooden framed cages covered with a fine mesh, as explained above to tomato plants and coriander, to obtain a stock rearing.

The gender of the collected *C. sanguinea* was determined and couples were maintained inside transparent plastic pots (500 mL) covered with a fine mesh in a climate chamber (25 ± 2 °C, 70 ± 10% r.h. and 13 h of light). They were fed with aphids, *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs, water and honey. When eggs were observed inside the pots, adults were removed and transferred to other pots. After egg hatching, the larvae were separated and fed with the same diet offered to the adults. Offspring from field-collected individuals were used only in the survivorship experiment and to evaluate the oviposition patterns of *C. sanguinea* (explained below). To all other experiments field-collected individuals were used.

Olfactometer experiments

To assess the role of odors from coriander plants in the vegetative stage in the *C. sanguinea* foraging behavior, we performed two-choice bioassays using a tubular Y-shaped olfactometer, modified from Sabelis and Van de Baan (1983). The olfactometer was 27 cm long and 3.5 cm wide. Plastic pots containing coriander or tomato plants were placed inside glass boxes (50 x 36 x 43 cm) and served as odor sources. The boxes were connected with the olfactometer arms with plastic hoses connected to valves that controlled the airflow. Airflow through both arms of the Y-tube was calibrated with a digital flow meter with valves between the air outlet of the containers of the odor sources and the arms of the olfactometer. The olfactometer basis was connected to a vacuum pump to produce an airflow that carried the volatiles from the glass boxes into the olfactometer. The airflow was adjusted to 0.45 m/s in each olfactometer arm. This speed was found to be suitable for studying *C. sanguinea* foraging behavior using the olfactometer in a pilot experiment.

Cycloneda sanguinea females were separated from males and were left without food for twenty-four hours before bioassays. We assumed that starved and mated females are responsible to find not only suitable patches for reproduction but also for feeding. Each female was observed individually in the olfactometer. To accomplish this, a plastic tube containing a female was connected to the olfactometer basis while the airflow was interrupted. When the individual entered into the olfactometer, the airflow were reestablished and the individuals tended to move towards an odor source. We considered that the observed individual made its choice when reached the end of the olfactometer arm and remained there for at least 30s. *Cycloneda sanguinea* is a highly mobile predator and we observed in previous bioassays that individuals moved among the odor sources before they take a decision (i.e. reach and remain at the end of the olfactometer arm). Each female was observed during at a maximum of 10 minutes or until they made a choice. In each bioassay, five replications were done and 16 individuals were observed per replication, totaling 80 individuals observed per bioassay. After observing four females, the odor sources were switched to the opposite arm of the olfactometer to avoid any bias related to any unforeseen asymmetry in the experimental set-up and the plants were chaged by others. When the observations ended, the olfactometer was cleaned with neutral detergent.

The following two-choice bioassays were performed in the Y-tube olfactometer: (*i*) coriander plants *vs.* clean air, (*ii*) tomato plants *vs.* tomato plants + coriander plants, (*iii*) tomato plants + coriander plants *vs.* coriander plants, (*iv*) coriander plants *vs.* aphid-infested tomato plants, and (*v*) aphid-infested tomato plants *vs.* aphid-infested tomato plants + coriander plants. Tomatoes plants were 30-40 days old and coriander plants 40-45 days old. Infested tomato plants were obtained by transferring 200 aphids from the stock rearing to a clean plant one week before the bioassays.

Oviposition of Cycloneda sanguinea *on coriander plants*

Coriander plants in the vegetative stage were attractive to *C. sanguinea* in the ofactometer bioassay. Therefore, we hypothesized that this attraction was due to these plants could be used by females as shelter and oviposition sites. Moreover, the plant architecture varies during the vegetative stage and these physical cues are also used by coccinellids to find oviposition sites (Evans, 2003). Thus, it is possible that the predator has preference for ovipositing on some stage of coriander development before flowering.

To test these assumptions, a release experiment was performed using three development stages of coriander plants. About 20 coriander seeds were directly sown in plastic pots (1 L) containing soil and a commercial plant substrate (Bioplant®) (proportion of 3:1) in a greenhouse. Three plastic pots containing coriander in different stages were placed inside an acrylic cage (60 cm x 60 cm x 80 cm) as follows: *i*) coriander in vegetative stage (35-40 days after germination) – in this stage the plant produces only leaves, *ii*) coriander near to the reproductive stage (45-50 days after germination) – in this stage the plant architecture changes and it becomes taller and *iii*) pre-blooming coriander (55-60 days after germination) – plant architecture is similar to the previous stage but plants have umbels with buds. Subsequently, one field-collected mated female of *C. sanguinea* was released inside the cage. Each replicate consisted of a cage with potted coriander plants in three development stages and one *C. sanguinea* female. A total of 30 replicates were done using this setup. Each individual was daily observed during three days to evaluate on which plant they were. After three days, the number of eggs per plant was counted. To ensure that females would have sufficient energy and stimulus for egg production, we offered tomato leaves infested with *M. persicae,* at a Petri dish, positioned in the center of the cages every day.

Ability of larvae to search for aphids

Because the coccinellids oviposited on coriander plants, we performed another experiment to evaluate if the hatched larvae on coriander plants were able to find aphid-infested tomato plants nearby coriander plants. To accomplish this, egg masses from field-collected adults were used. We used only egg masses with more than fifteen eggs from the same mother. These egg masses were positioned in the center of a circular arena and eggs were attached to a coriander plant. The day after egg hatching, it was counted the number of newly-hatched larvae. After that, we counted the number of larvae per plant in the arena once a day during three consecutive days in a free choice experiment. Thus, in this experiment the larvae could chose between the following plants in the arena after hatching: (*i*) Coriander with eggs – coriander plants where egg masses were attached in the middle of the arena; (*ii*) Clean coriander plants; (*iii*) Clean tomato plants and (*iv*) Aphid-infested tomato plants.

The plant *Coriander with eggs* served as a release area for the newly emerged larvae and it was positioned in the center of a circular arena with a radius of 30 cm. The other treatments were positioned 20 cm apart from the coriander with eggs in the arena and equally spaced from each other. The arena was recovered with a fine mesh before running the experiment to avoid herbivore contamination. In the treatments with coriander plants, five seeds were sown in the same place. Two tomato plants were kept without aphids and one tomato plant was infested with 150 aphids four days before the experiment beginning. The aphid-infested plant was isolated from others with a fine mesh to avoid infestation of other treatments with aphids. Thus, in the circular arena we had two uninfested coriander plants, two uninfested tomato plants and one aphid-infested tomato plant. All plants used in this experiment had 30 days after germination.

Survival of Cycloneda sanguinea *with coriander flowers*

The direct effect of coriander flowers (alternative resource) on the survival of *C. sanguinea* females was assessed using insects obtained from the laboratory rearing, following the methodology proposed by Amaral et al. (2013). Transparent plastic pots

(500 mL) were cut at the basis to form an entrance for coriander flowers into the pot. The upper side of the pot was recovered with a fine-mesh. All pots were secured with a wooden post fixed in the soil and close to the plant flowers (see Amaral et al., 2013 for further details). Newly emerged adults were sexed and fed with aphids, eggs of *A. kuehniella* and honey during 48 hs. In this way, we reduced the natural mortality of the adults due to starvation; consequently, effects that were not related to the treatments were avoided (Amaral et al., 2013). After this period, new pairs were formed and introduced into the plastic pots.

The survival of *C. sanguinea* females was observed during 40 days in the following treatments: (*i*) negative control – moistened cotton wool; (*ii*) positive control –aphids; (*iii*) flowers only – moistened cotton + coriander flowers; (*iv*) flowers and aphids – moistened cotton + coriander flowers + aphids. In all treatments moistened cotton wool was placed in the pot and it was replaced daily to avoid fungal contamination. In the treatments with aphids (*M. persicae*) they were offered *ad libitum* to all individuals by daily placing aphids into the pot. Coriander flowers were replaced after two days to ensure the availability of pollen and nectar. In the control groups (positive and negative) coriander flowers were cut but the flower stalks were maintained inside the plastic pots. A total of 30 replicates per treatment were done.

Statistical analyses

Olfactometer data were analyzed using a Generalized Linear Model (GLM) with a Poisson error distribution. The analyses were performed separately for each double-choice bioassay. We used the choice as response variable and the interaction among treatment choice, the treatment position in the olfactometer in each observation and the replication as explanatory variables. These two latter explanatory variables were included in the model to verify if there was any bias due to treatment position during the observations and among replicates. If no significant interaction among these variables were observed they were removed from the model and a new model was fitted. This new model was compared with the full model by an Analysis of Deviance using a Chi-square test (Crawley, 2007). The analysis continued from simplest model. After that, it was verified if the individuals' choice were affected by one of the two odor sources that they were exposed in each bioassay testing the significance of the variables by using a Chi-square test followed by a model residual analysis (Crawley, 2007). The final model was compared with a null model by an Analysis of Deviance with a Chi-square test. If significant differences from the null model were achieved we accepted the final model (Crawley, 2007).

To analyze whether *C. sanguinea* females had preference to remain or to lay eggs on coriander plants in different development stages we first summed the number of choices and the number of eggs per treatment during the three days of evaluation. After that, we fitted a GLM analysis with a binomial error distribution. The number of choices per treatment per female / total choices per female and the number of eggs per treatment per female / total eggs per female were used as response variables and the treatment was used as explanatory variable. To test the significance of variables in the model we used an Analysis of Deviance with a Chi-square test, followed by a model residual analysis (Crawley, 2007). If there was some overdispersion in our data we fitted a new model using the quasibinomial error distribution and repeated all the procedure described above, including the model residual analysis. The differences among treatments were evaluated by a model contrast analysis (Crawley, 2007). To test if there was a relationship between the proportion of adults per coriander development stage and proportion of eggs on these plants, we fitted a linear model using the total number of eggs per plant per female as response variable and the total number of choices per female as explanatory variable.

The experiment on larval choice in the circular arena was also evaluated using a GLM but with a binomial error distribution and using the same procedure adopted to evaluate the females' choices and oviposition pattern experiments described above. In this experiment we performed the analyses separately for each day of observation, because the larval behavior was different in each day of experiment. We then used the number of larvae per treatment in each day / total number of larvae in all treatments as response variables and the total number of larvae in the experiment per day as response variables.

A Kaplan-Meier test was performed to assess the effects of different diets on *C. sanguinea* survival. First, we estimated the survival curves for females exposed to each treatment using the Kaplan-Meier analysis, adjusted for a non-linear model identity with censored data (Crawley, 2007). To assess the model significance it was performed a Log-rank test with all variables included. All possible comparisons between treatments were performed using the Log-rank test corrected for multiple comparisions. Adopting this procedure, it was possible to distinguish all possible differences among treatments. After that we also analyzed the number of eggs per female per day and eggs fertility on these treatments. We performed two separated analysis for each variable and fitted a Repeated Measures Analysis of Variance (RM-ANOVA), using the females identity as repeated measures (Crawley, 2007). All statistical analyses were performed using the Software R v 2.15.0 (R Development Core Team, 2010).

Results

Olfactometer experiments

Coriander volatiles were attractive to *C. sanguinea* females (Figure 1). In the first bioassay, females preferred coriander volatiles instead of clean air ($\chi^2 = 1.89$, d.f. = 4, $P = 0.007$) (Figure 1). When the females were given a choice between volatiles from tomato plants and volatiles from tomato plants plus coriander plants, we observed a significant preference for volatiles from tomato plants and coriander plants together ($\chi^2 = 0.37$, d.f. = 4, $P < 0.001$) (Figure 1). Attraction by these volatiles was confirmed in the third bioassay where it was offered a choice of coriander volatiles against tomato plants plus coriander plants volatiles in Y-tube experiment. There was no preference between volatiles from coriander plants and tomato plants plus coriander plants ($\chi^2 = 1.21$, d.f. = 4, $P = 0.3707$) (Figure 1).

When volatiles from aphid-infested tomato plants were tested against volatiles from coriander plants, females of *C. sanguinea* preferred those from aphid-infested plants ($\chi^2 =1.13$, d.f. = 4, $p < 0.0001$) (Figure 1). However, volatiles from coriander plants together with volatiles from aphid-infested tomato plants were more attractive to *C. sanguinea* females than just volatiles from aphid-infested plants ($\chi^2 = 2.18$, d.f. = 4; $p < 0.0001$) (Figure 1).

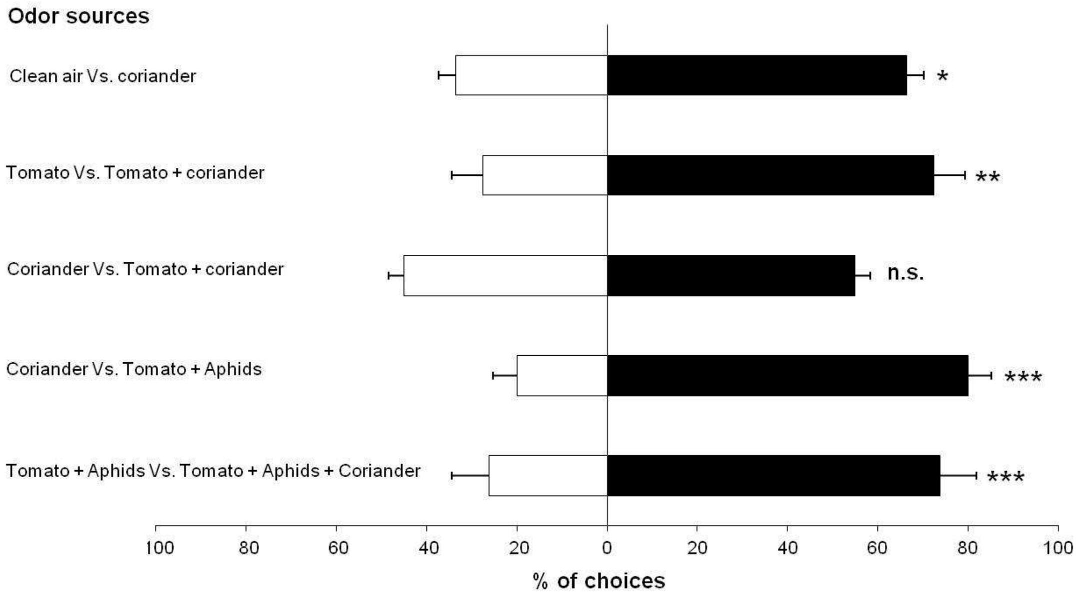

Figure 1 – Two choice tests with mated females of *Cycloneda sanguinea* in a "Y" tube olfactometer with different combination of odor sources. * indicates significant differences between treatments at $P < 0.05$ ** indicates significant differences between treatments at $P < 0.001$, *** indicate significant differences between treatments at $P < 0.0001$ and "n.s." indicate non significant differences ($P > 0.05$). Each bar represents the average number (± SE) of predators observed in each two choice test. Five replicates of each two choice test were done (n = 80 individuals observed per two choice comparision).

Oviposition of C. sanguinea *on coriander plants*

The plant choice of *C. sanguinea* females was significantly affected by the development stage of the coriander plants ($\chi^2 = 166.28$, d.f. = 87, P = 0.0009). Females preferred to stay more on pre-flowering plants (55 – 60 days after germination) than on the other plant stages (Figure 2). However, adult plant choice did not result in females laying more eggs on these plants and no relationship between these variables was achieved ($R^2 = -0.007$, F = 0.43, d.f. = 88, P = 0.515) (Figure 3). Females left similar proportion of eggs on coriander plants of all development stages ($\chi^2 = 1124.5$, d.f. = 74, P = 0.649) (Figure 3).

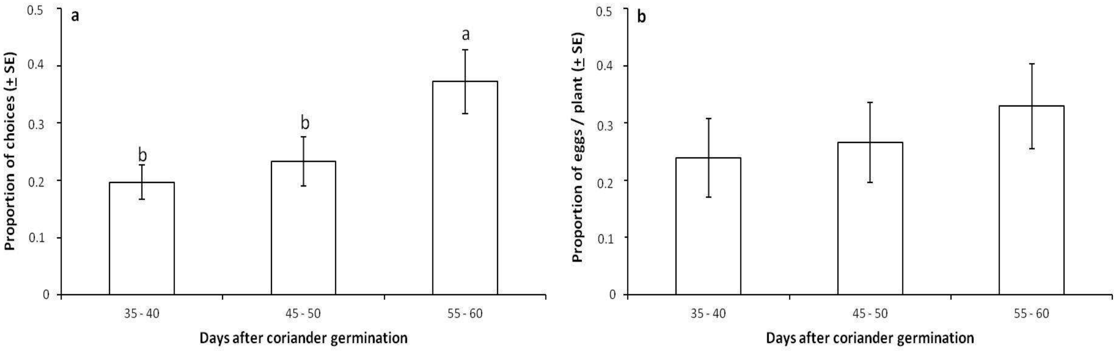

Figure 2 – **(a)** Proportion of mated *Cycloneda sanguinea* females visiting coriander plants and **(b)** proportion of eggs per female per coriander plant in different development stages before blooming and during three days in a free-choice experiment. Means followed by the same letter did not differ significantly ($P < 0.05$).

Ability of larvae to search for aphids

When the *C. sanguinea* larvae hatched on coriander plants, they were able to find aphid-infested tomato plants. Nevertheless, they tended to move among plants along the three days of observation (Figure 3). On the first day, the larvae preferred to stay mostly on coriander plants where they hatched rather than the other plants (χ^2 = 244.08, d.f. = 104, $P < 0.0001$) (Figure 3). On the second day, the larvae moved to other plants and similar proportion of larvae per plant were found in all plants except on the uninfested tomato plants, where proportionally fewer larvae were found (χ^2 = 153.13, d.f. = 116, $P < 0.0001$) (Figure 3). During the third day the larvae continued moving between plants and proportionally more individuals were found on aphid-infested tomato plants than on other uninfested plants (χ^2 = 138.42, d.f. = 92, $P < 0.0001$) (Figure 3).

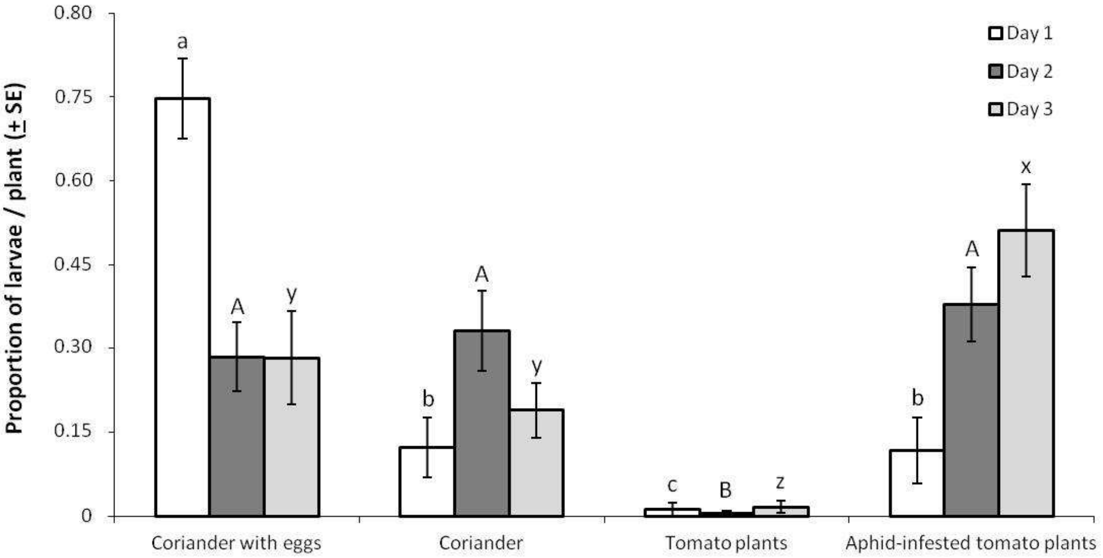

Figure 3 – Proportion of *Cycloneda sanguinea* larvae visiting different plants in a circular arena during three days after egg hatching, based on the total number of larvae per treatment / total number of larvae observed in each day. Eggs were placed on coriander plants positioned in the center of the arena ("Coriander with eggs"). Means followed by the same capital or lower case letters did not differ significantly (P > 0.05) within each day of observation.

Survival and oviposition of C. sanguinea *with coriander flowers*

The survival of *C. sanguinea* females was affected by the diet that they were exposed (Log-rank = 131.00, d.f. = 3, P < 0.0001) (Figure 4). Females fed with coriander flowers lived longer (7.77 ± 0.49 days) than the control group (4.23 ± 0.36 days) (Log-rank = 23.30, d.f. = 1, P < 0.0001) but had a lower survival than the females fed with aphids (19.86 ± 1.95 days) (Log-rank = 32.00, d.f. = 1, P < 0.0001) and with aphids plus coriander flowers (25.89 ± 1.77) (Log-rank = 43.40, d.f. = 1, P < 0.0001) (Figure 4). Indeed individuals fed with aphids (Log-rank = 56.4, d.f. = 1, P < 0.0001) and aphids plus flowers (Log-rank = 32.00, d.f. = 1, P < 0.0001) differed significantly from the control group. However, females fed with aphids plus flowers lived longer than those fed with just aphids (Log-rank = 4.30, d.f. = 1, P = 0.0384) (Figure 4).

Females did not lay eggs in the control groups and when they were fed only with coriander flowers. Due to this, we did not include these data in our analysis. Nevertheless, the number of eggs per female per day (F = 0.474, d.f. = 1, P = 0.492) and egg fertility (F = 0.013, d.f. = 1, P = 0.909) did not differ significantly if they were fed with aphids or aphids plus coriander flowers (Figure 5).

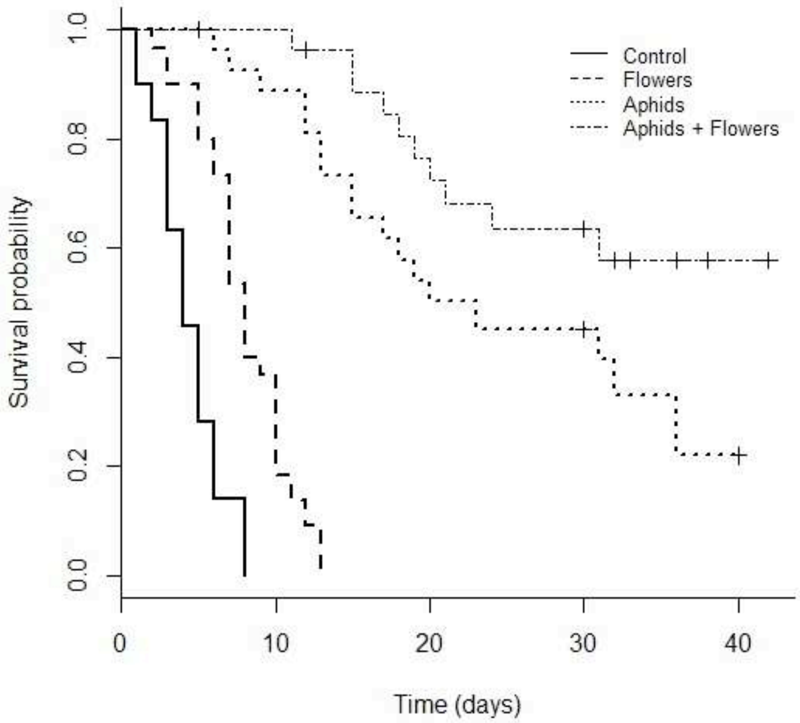

Figure 4 – Kaplan-Meier estimates of survival of newly-emerged *Cycloneda sanguinea* females that were fed with water (negative control), flowers of coriander plants, aphids *Myzus persicae* (positive control) and aphids plus coriander flowers. "+" indicates censored data.

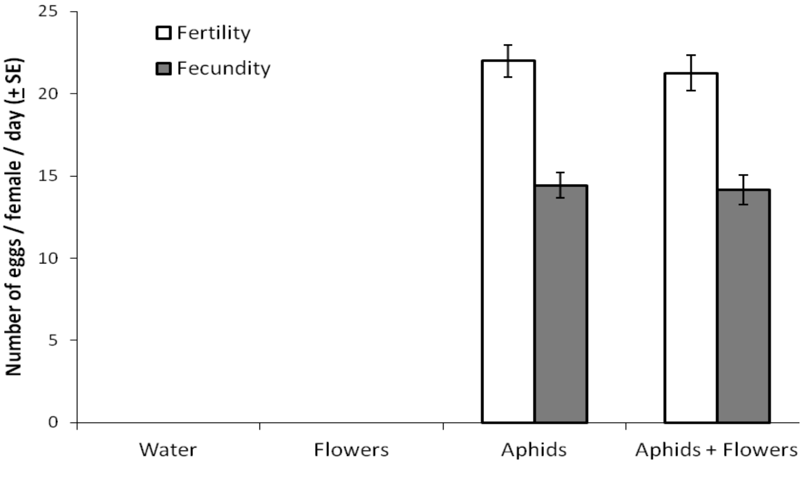

Figure 5 – Fertility and fecundity of *Cycloneda sanguinea* females fed with water (negative control), coriander flowers, Aphids *Myzus persicae* (positive control) and Aphids plus coriander flowers. Note that no females oviposited when fed with water and coriander flowers and due to this these data were not computed in the statistical analysis. Fertility and fecundity data were compared separately.

Discussion

Cycloneda sanguinea recognized and moved towards coriander volatiles, and this attraction was not affected by volatiles of tomato plants. This can partially explain why this predator is more abundant in tomato crops intercropped with coriander than tomato plants in monoculture even when coriander is in the vegetative stage. However, attraction by undamaged plants is not so common among coccinellids (Sarmento et al., 2007; Petterson et al., 2008) but some publications suggest that volatiles from specific plants can be attractive. For example, odors from barley plants mixed with weeds can be more attractive for *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) than barley plants only (Ninkovic and Petterson, 2003). Schaller and Nentwig (2000) tested the attraction of 22 uninfested plants to *C. septempunctata* and found that only two plants were attractive in a Y-tube olfactometer. All plants that were attractive to these coccinellids have in common that they are used as a (alternative or not) resource, can harbor aphids or act as arrestant stimulus together with other plants.

Volatiles from aphid-infested tomato plants were more attractive to *C. sanguinea* than coriander volatiles probably because aphids represent an immediate essential food source for coccinellid females and their offspring (Evans, 2003; Obrycki et al., 2009). Additionally, coriander volatiles mixed with volatiles from aphid-infested tomato plants were even more attractive than aphid-infested tomato plants alone. To explain this pattern it is necessary to consider two main points. First, the evolution of food preference and transitions among food types (*e.g.* aphidophagy, pollen-feeding, micophagy) seems to shape the evolution of Coccinellidae (Giorgi et al., 2009; Magro et al., 2010). Aphidophagy evolved before pollen-feeding in this group but pollen-feeding may have played a role in Coccinellidae subfamily diversification in some instances (Giorgi et al., 2009). Probably, generalist coccinellids that also feed on plant-provided food, such as *C. sanguinea*, evolved the capacity of recognizing plants with edible pollen and nectar. Second, *C. sanguinea* has many natural enemies such as parasitoids (Riddick et al., 2009) and habitats with higher botanical diversity could provide more possibilities to escape from natural enemies and other antagonists (Janssen et al., 2007; Tixier et al., 2013). Due to the life-history of coccinellids with plants and antagonists, coccinellids may have learned how to use plants in more diversified habitats in other ways such as shelter and oviposition sites, because it could be an adaptative behavior.

Our study clearly demonstrated that *C. sanguinea* use coriander plants as oviposition sites and this is not related with plant age before flowering. When females find a suitable patch for oviposition within a habitat they need evaluate the resource availability (aphids) and the predation risk that their eggs and offspring are exposed (Evans, 2003). Probably, patches with coriander plants near an aphid source can support more coccinellids and their offspring than simple habitats, such as monocultures, by reducing the predation risk due to a higher botanical diversity. In fact, when tomato plants (Togni et al., 2009, 2010) and kale (Resende et al., 2010) were intercropped with coriander in the field, there was a higher diversity and abundance of coccinellids, including *C. sanguinea*, than tomato plants or kale planted in monocultures.

However, the egg distance from the aphid source imposes a trade-off between the potential progeny starvation if eggs are too far from the aphid source and the risk of predation by other species if is too close (Hodek and Evans, 2012). In our experiment *C. sanguinea* hatched larvae in coriander plants were able to search to aphid-infested tomato plants 20 cm apart. During the first day after hatching, most larvae remained aggregated and the first hatched larvae cannibalized sibling eggs. Cannibalism can benefit the cannibals fitness because neonate larvae hve limited mobility and feeding on siblings provides a high quality source of energy for further foraging for aphids and eliminate potential competitors (Ferran and Dixon, 1993; Michaud, 2003). In the second day most larvae started the dispersion within the arena. Proportionally, more individuals were found in the aphid-infested tomato plants and coriander plants, probably as a result of a more active foraging behavior than the first day (Ferran and Dixon, 1993; Hodek and Evans, 2012). Nevertheless, on the third day more larvae found the aphid-infested tomato plants and fed on their prey, most likely following the larval tracks from other individuals (Hemptinne et al., 2000; Meisner and Ives, 2013). If *C. sanguinea* females use coriander as oviposition sites, their offspring can contribute to aphid biological control, mostly after three days when larvae are more active and more individuals can find the aphid sources.

Investigating whether coriander plants are used as oviposition sites we observed adults more frequently in plants near flowering (55 – 60 days after germination). Coccinellids can use visual besides olfactory cues to search for suitable patches within a habitat (Hodek and Envans, 2012). Coriander architecture near bloom is very similar to coriander flowering with exception that no resource is available. After 60 days the flowers start opening and soon pollen and nectar are available to coccinellids. Possibly females preferred to use coriander as shelter when plants are near flowering due to their similarity with flowering plants.

After opened, coriander flower morphology and plant architecture ensure accessibility of resources for many species, including coccinellids (Patt et al., 1997). In fact, *C. sanguinea* can feed on coriander flowers and increase their survivorship. Combined, these facts suggest a life-history association between coriander plants and *C. sanguinea* that should be considered when selecting plants for intercropping aiming coccinellid attraction. For example, Amaral et al. (2013) observed that pollen and nectar from *Bidens pilosa* L. and *Agerantum conyzoides* L. (both Asteraceae) – two South American native weeds – increased *C. sanguinea* adults and larvae survivorship while did not affected the survivorship of the exotic coccinellid *H. axyridis*. We also can conclude that coriander flowers are alternative resources to *C. sanguinea* because an aphid diet can support higher adult survivorship than only coriander flowers. But when aphids are available, coriander flowers can supplement female diet and they can live longer, contributing for predator attraction, retention and functionality in aphid's biological control in many agroecosystems (Symondson et al., 2002; Lundgren, 2009; Obrycki et al., 2009; Hodek and Evans, 2012). Anyway, apparently, the nutrients and energy provided by coriander flowers were not allocated to the reproduction of *C. sanguinea*; Females oviposited only if aphids were available and no differences were detected when supplementing their diets with coriander flowers. Similarly, Choate and Lundgren (2012) also did not find effects on reproduction and oocytes volume of *C. maculata* fed with a mixed diet of aphids and extrafloral nectar from *Vicia faba.* Non-prey foods usually have a low positive effect on adult performance and reproduction, but this effect is highly variable among studies (Lundgren, 2009). Therefore, supplementing coccinellid diets with non-prey foods should consider the pollen and nectar quality for different assemblages of coccinellids.

In conclusion, we demonstrated that *C. sanguinea* use odors from damaged and undamaged plants to assess habitat quality. This is directly related to benefits for adults, eggs and larvae. A habitat with coriander plants represents more oviposition sites to adults at the same time that larvae hatched on coriander plants can find nearby aphid sources. Probably a long life-history with coriander plants and antagonists shaped these traits in *C. sanguinea* and further investigations considering multiple hypotheses to explicitly test this idea should be done. Regarding biological control we partially elucidated why coriander plants can attract *C. sanguinea* and probably other generalist predators. Coriander plants are attractant to *C. sanguinea* because the plant at different development stages can benefit the predator adults and their offspring in different ways (shelter, oviposition sites and food). Intercropping coriander with tomato plants can attract this predator before prey arrival or retain the predator in the area when aphids are available, directly benefiting adults and their offspring. Furthermore, if growers left some coriander plants for flowering interspersed with tomato plants it can increase predator survival and prolong the effect of each predator on biological control. Further investigations to understand the common mechanisms involved in the attraction and retention of aphidophagous coccinellids is needed in order to find ways to manipulate such interactions and contribute to maintain the ecosystem service of biological control.

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GENERAL CONCLUSIONS

Factors at different spatial scales significantly affect conservation biological control in organic crop systems. Different cropped and non-cropped habitats should be maintained in the farm level. Such habitats have great potential to conserve and maintain many natural enemy species in the farm and also contribute to a rapid colonization of new habitats. Therefore, conservation of beneficial insects such as natural enemies is the first step to favor biological control in organic farms. Nevertheless, different habitat types should provide adequate resources and conditions in order to qualitatively (number of species) and quantitatively (abundance of individuals) maintain natural enemies in the farm level.

The colonization of habitats by natural enemies is not sufficient for a positive effect of biological control. The diversity of habitats in the farm, diversity of vegetation and different pest management strategies interact and can directly affect biological control efficiency, as demonstrated here for *Bemisia tabaci.* Thus, designing farms with a higher diversity of habitats and resource for natural enemies should be done considering that the adverse factors such as insecticide spraying should be avoided. Considering the diversity of habitats and resources and avoiding adverse factors can turn biological control a more reliable and constant ecosystem service which is directly related to the conservation of diverse communities of natural enemies in organic farms.

Case-specific interactions, as demonstrated her for the interaction between coriander and *C. sanguinea*, need to be studied in deep because predator species can differently respond to habitat diversification and this is directly associated with predator attraction and retention. When considering a plant species for intercropping purposes, the interactions of predator species with the introduced plant can reveal other interactions between the plant and natural enemies, beyond the provision of plant-provided food. Plants that predators use for increasing their survivorship and their offspring could be considered the best candidates for introduction. Here, coriander plants showed many positive characteristics to be intercropped with tomato plants in order to attract and retain predatory coccinellids that can improve biological control of aphids.

Conservation biological control strategies should be implemented considering factors that operate at different spatial scales. The species responsible for biological control need to be conserved, using strategies that consider spatial scales beyond the plot level. The functionality of these beneficial organisms in agroecosystems, mainly in the organic systems, depends on the conservation of different species in the farm level, management of local habitats, manipulation of local interactions with introduced resources and the interaction between species. Togheter, these strategies can contribute to design more sustainable agricultural landscapes where the needs of food production are integrated with the needs for biodiversity conservation.

APPENDICES

CHAPTER I

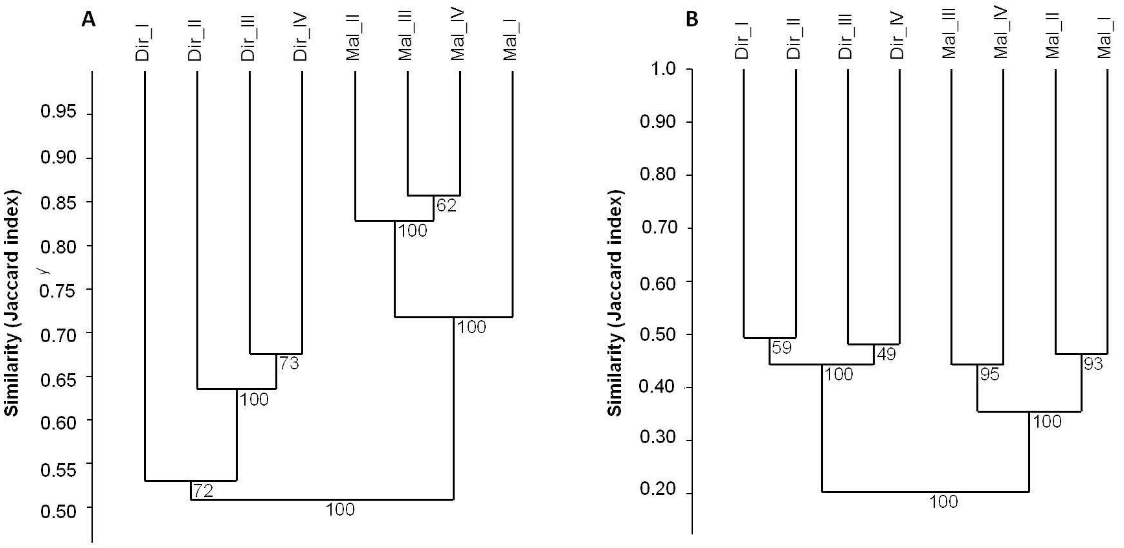

Figure A1 – – Similarity in species identity of herbivore (A) and predator (B) communities using Malaise traps (Mal) and direct sampling of the insects at the plant (Dir) at four different sample sites in the Brazilian Federal District, Brazil, from March/2012 to February/2013. Cophenetic correlation coefficient for herbivores = 0.933 and for predators = 0.914. Number above each ramification indicates the consistence of nodes based on the bootstrap procedure with 100 randomizations. Note that 'y' axes are at different scales.

Methods: The hierarchical clustering analysis was used to show how predator and herbivore communities on different sample site clustered through the unweighted pair-group average (UPGAM) method calculated using the Jaccard index. A bootstrap of 100 randomizations was performed to test the consistency of the nodes in the dendrogram. The cophenetic correlation coefficient was used to test the goodness of fit of the dendrogram in preserving the pairwise distances between the original unmodeled data points.

CHAPTER II

Table A1 – Description of 20 small farms cropping tomatoes where whiteflies and their natural enemies were sampled in the Brazilian Federal District, Brazil, 2013.

Farm	Management system	Wind breaks	Agroforestry	Crop species	Crops surrounding plot	Diversity of plants within plot	Weed management	Irrigation	Soil cover	Fertilization	Pest control	Disturbance level	Diversity level
I	Conventional	No	No	Kale, capsicum, cucumber, eucalyptus	Eucalyptus, cucumber	Monoculture	Herbicide	Drip	No	Exclusively Chemical	Broad spectrum insecticides and Bt based products in the pre harvest period; At least three times a week	**High**	**Very low**
II	Conventional	No	No	Kale, eggplant, capsicum, jilo	Eggplant, capsicum	Monoculture	Herbicide	Drip	No	Exclusively Chemical	Broad spectrum insecticides; Three to four times a week	**High**	**Very low**
III	Conventional	No	No	Capsicum, eggplant	Eggplant	Monoculture	Herbicide	Drip	Plastic mulch	Exclusively chemical	Broad spectrum insecticides; At least twice a week	**High**	**Very low**
IV	Conventional	No	No	Capsicum, maize, cucumber	Capsicum	Monoculture	Herbicide	Drip	No	Chemical and chicken manure	Broad spectrum insecticides; Twice a week	**High**	**Very Low**
V	Conventional	No	No	Lettuce, Eggplant	Lettuce	Monoculture	Herbicide	Drip	No	Chemical and chicken manure	Broad spectrum insecticides; At least three times a week	**High**	**Very Low**

VI	Transition to organic	No	No	kale, strawberry, maize, pumpkin, papaya, broccoli	Pumpking, strawberry	Monoculture	Manual weeding, sometimes herbicide use; weeds surrounding the plot	Sprinkler	Plastic mulch	Tillage and chicken manure	Neem and other botanical insecticides, Bordeaux mixture and other mixtures; Twice a week	**Moderate**	**Low**
VII	Transition to organic	No	No	kale, basil, marjoran, maize, broccoli	No (fallow ground)	Monoculture interspersed with a few weeds	Manual weeding with moderate frequency	Sprinkler	Very sparse living cover with weeds	EM-Bokashi and chicken manure	Bt based products and botanical insectides; Once a week	**Moderate**	**Low**
VIII	Transition to organic	Yes; one windbreak with elephant grass dividing the total area	No	Lettuce, strawberry, kale, coriander, broccoli, cucumber, capsicum	Lettuce and kale	Monoculture	Manual weeding every week	Drip	Plastic mulch	EM-Bokashi and chicken manure	Bt based products, biological control with fungus, Bordeaux mixture and *Trichogramma* sp.; Twice a week	**Moderate**	**Low**
IX	Transition to organic	Yes; few windbreaks with elephant grass and mexican sunflower interspersed with crops	No	Cucumber, kale, pea, strawberry, maize, broccoli, carrot, roquette, beet	Kale, cucumber, pea	Monoculture	Manual weeding every week	Drip	Very sparse living cover with weeds	EM-Bokashi, organic compost with chicken manure and natural termophosphate	Neem based products and other botanical insecticides, different mistures but mainly Bordeaux mixture, lime sulfurand Bt based products; Once a week	**Moderate**	**Low**

X	Transition to organic	No	No	Broccoli, maize, jilo, inhame, kale, watercrass, pumpkin	Pumpkin	Monoculture	Manual weeding every week	Drip	Mulching with weeds and grass	EM-Bokashi, organic compost with chicken manure	Neem based products, Bordeaux mixture, *Trichogramma* sp.; Fortnightly	**Moderate**	**Low**
XI	Organic	Yes; elephant grass, citrus species, banana and mango	No	18 crop species	Pea, maize, cabbage, banana	Interspersed and surrounded by rows of weeds and in polyculture with cabbage	Manual and selective removal of weeds; weeds are removed in alternated rows and at different weeks and total removal only in the basis of tomato plants	Sprinkler	Living ground cover with weeds	Organic compost with chicken manure and natural termophosphate	Homeopathy and Bordeaux mixture; According sample plans; Only when is needed	**Low**	**Moderate**
XII	Organic	Yes; papaya, banana, citrus and two species of grass	No	17 crop species	Strawberry, kale, cabbage	Interspersed and surrounded by rows of weeds	Selective removal of weeds	Sprinkler	Living ground cover with weeds	Organic compost with chicken manure and natural termophosphate	Bordeaux mixture, lime sulfur and entomopathogenic fungus; Mixtures: two times during all crop cycle; Fungus: only when insect pest outbreaks occur	**Low**	**Moderate**

XIII	Organic	Yes; banana, mexican sunflower, elephant and napier grass	No	12 crop species	kale, pumpkin, banana, okra, sugar-beet	Interspersed and surrounded by rows of weeds and in polyculture with cabbage	Manual and selective removal of weeds; weeds are removed in alternated rows and at different weeks and total removal only in the basis of tomato plants	Sprinkler	Living ground cover with weeds	Organic compost with chicken manure, natural termophosphate and EM-Bokashi	lime sulfur, Bordeaux mixture and *Trichogramma* sp.; When needed	**Low**	**Moderate**
XIV	Organic	Yes; many native species, mexican sunflower, grass and castor bean	No	14 crop species	Carrot, coriander, cofee and onion	Polyculture with coriander and carrot; surrounded by rows of weeds	Selective removal of weeds	Sprinkler	Moulching with weeds and grass	Organic compost with chicken manure, EM-Bokashi, natural termophosphate and bone meal	Lime sulfur; When needed	**Low**	**Moderate**
XV	Organic	Yes; banana, mango, papaya, castor bean	No	11 crop species	Lettuce, chili-pepper, coriander, kale, papaya	Polyculture with coriander, lettuce and kale; some rows of weeds surrounding plot area	Selective removal of weeds	Sprinkler	Moulching with grass	Organic compost with chicken and cattle manure	Lime sulfur, bordeaux mixture and neem based products; When needed	**Low**	**Moderate**
XVI	Organic / agroecological	Yes; banana, citrus, fruticulture crops, native plants, elephant grass, papaya and cofee	Yes; agroforestry "islands" interspersed with crops and one agroforestry in continuum with natural areas	More than 20 crops	Carrot, dill, lettuce, coriander, garlic, onion, basil; surrounded by windbreaks	Polyculture with onion, coriander, garlic and carrot	Selective removal and used as mulch	Drip	Living ground covers and mulching with weeds and grass	Green manure before planting; organic compost with chicken manure, EM-Bokashi	Natural	**Very low**	**High**

XVII	Organic / agroecological	Yes; rows of agroforestries dividing the area and surrounding crop areas	Yes; agroforestry (> 10 year old) in continuum with natural areas	More than 20 crop species	pea, kale, papaya, citrus	Tomato plants interspersed with weeds, papaya, native trees; plot surrounded by rows of weeds	Selective removal and used as mulch	Sprinkler	Living ground covers and mulching with weeds	Green manure before planting; organic compost with chicken manure, EM-Bokashi and fish compost	*Trichoderma* spp.; Before planting; natural pest control during crop cycle	**Very low**	**High**
XVIII	Organic / agroecological	Yes; rows of agroforestries dividing the area and surrounding crop areas	Yes; rows of agroforestries dividing the area and surrounding crop areas; Agroforestry in continuum with natural areas	More than 20 crops	Lettuce, garlic, kale, onion, yellow sweet potato, passion fruit and chive	polyculture with yellow sweet potato, interspersed with weeds; rows of weeds surrounding the plot	Selective removal and used as mulch	Sprinkler	Living ground covers and mulching with weeds and grass	Green manure before planting; organic compost with chicken manure, EM-Bokashi	Natural	**Very low**	**High**
XIX	Organic / agroecological	Yes; rows of banana interspersed with citrus, mexican sunflowers and newly emerged native species; windbreaks with elephant grass around the planting area	Yes; rows of early stage agroforestries (<2 years old) interspersed with crops and windbreaks. In the farm werst side there is an agroforestry (8 years old) in continuum with natural areas	More than 20 crops	Strawberry, broccoli, eggplant, coriander, capsicum and surrounded by windbreaks	Polyculture with broccoli and lettuce; interspersed with rows of banana, mexican sunflowers and some citrus species; rows of weeds around the plot	Selective removal and used as mulch	Drip	Living ground covers and mulching with weeds and grass	Green manure before planting; organic compost with chicken manure, EM-Bokashi and natural termophosphate	Natural	**Very low**	**High**

XX	Organic / agroecological	Yes; agroforestry interspersed with banana, citrus and other frutiferous crops dividing vegetable crops area	Yes; the entire farm is divided with agroforestries in continuum with natural areas	More than 20 crops	Lettuce, kale, broccoli, inhame and eggplant; weed rows; surrounded by windbreaks	Interspersed with many weeds and in polyculture with lettuce and kale; rows of weeds around the plot	Selective removal and used as mulch	Sprinkler	Living ground covers and mulching with weeds and grass	Green manure before planting; organic compost with chicken manure, EM-Bokashi and tillage	Natural	**Very low**	**High**