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Influence of cover crops on natural enemies
diversity in Citrus plantations

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Abstract

The interest about conservation biological control has been increasing markedly over the past two decades. It is believed that the appropriate cultural methods can lead to pest control which doesn't harm environment as conventional pesticides do. The aim of this study was to compare the influence of cover crop type and the distance from hedges on the abundance and the diversity of natural enemies, which are the key factor in biological control. There were selected 18 plots in an ecologically grown orchard southern of the city of Valencia. Aspiration was chosen as the appropriate method of sampling and was carried out 18 times during one year. Shannon's and Weaver's diversity indices, commonly employed by many scientists, were used for the comparison of diversity between plots with different cover crops and different distances from the hedges. There were slight indications that the presence of alfalfa, *Medicago sativa L.*, influences positively the abundance and the diversity of natural enemies. The strongest form of this effect was observed within tree layers. However, the variances between different types of cover crops were not as considerable as it was expected. The influence of the hedges was not confirmed at all, which was in contradiction with many recent studies. Commonly used Jaccard's and Sorensen's coefficient of similarity, were employed for the comparison of plots with different cover crops and different distance from the hedges. The plots with similar plant species compositions showed the highest similarities in species composition of natural enemies. Hence, it supports the idea that the cover crop composition strongly influences the diversity of natural enemies.

Abstrakt

V posledních dvaceti letech se výrazně zvyšuje zájem o biologickou ochranu rostlin pomocí takzvané konzervace. Věří se, že správné vybrané zemědělské techniky mohou vést k úspěšné ochraně rostlin před škůdci, i bez použití pesticidů, které poškozují životní prostředí. Cílem této práce bylo porovnat vliv křicích plodin a vzdálenosti od živého plotu na hojnost a různorodost přírodních nepřátel škůdců, kteří jsou v biologické ochraně rostlin klíčovým faktorem. K tomuto účelu, bylo vybráno 18 parcel v ekologickém citrusovém sadu jižně od města Valencie. Jako metoda byla zvoleno odsávání a bylo provedeno 18 sběrů vzorků v průběhu jednoho roku. Pro porovnání různorodosti mezi jednotlivými parcelami s rozličnými krycími plodinami a rozdílnou vzdáleností od živého plotu byli použity často využívané indexy různorodosti dle Shannona a dle Weavera. Ačkoli byli rozdíly mezi hodnotami indexů menší než se očekávalo, byly pozorovány jemné náznaky, že vojtěška, *Medicago Sativa* L., pozitivně ovlivňuje hojnost a různorodost přírodních nepřátel škůdců. Tento efekt byl nejzřetelnější u vzorků získaných ze stromů. Vliv vzdálenosti od živého plotu se zdaleka neprojevil tak, jak se očekávalo. Hojnost ani různorodost přírodních nepřátel škůdců nebyla vzdálenosti od živého plotu závislá. Toto bylo v rozporu s mnoha předchozími studiemi. Pro srovnání podobnosti druhového zastoupení přírodních nepřátel byly použity koeficienty podobnosti dle Jaccarda a dle Sorensena. Rozdíly mezi hodnotami byly dosti malé. Bylo však pozorováno, že krycí plodiny s podobným druhovým zastoupením přispívají k druhové podobnosti přírodních nepřátel škůdců. To podporuje myšlenku, že má kompozice rostlinných druhů značný význam na druhovou kompozici přírodních nepřátel škůdců.

Declaration

I hereby declare, that the whole thesis is my original work and that I have not received outside assistance, except where explicitly stated otherwise in the text or in the bibliography. I declare that it has not been submitted in whole, or in part, for any other degree.

In Prague,

Signature:

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1. Introduction

Agriculture has been altering environment and biodiversity since the people started to use first plants and animal species for our needs (Briggs and Courtney, 1989). However the extension of croplands increased during the past 300 years by 466% up to 12% of the terrestrial land to the prejudice of the natural ecosystems (Altieri and Nicholls, 1994; Klein Goldewijk and Ramankutty, 2004; (Leff *et al.*, 2004)). The doubling of agricultural production during the past 35 years has been associated with the implement of high amount of fertilizers, herbicides, pesticides and fungicides which the alteration of environment amplifies. It has been resulted in eutrophication and contamination of all ecosystems in the world. This factors and especially the eutrophication together with the fragmentation and destruction of habitats are considered to be ones of the main factors determining among others the loss of biodiversity and negative impacts on the complex ecologic functions and environmental sustainability (Tilman, 1999; Alan *et al.*, 2002; Benton *et al.*, 2003).

Spain is one of the world's largest producers of citruses. Citrus farms occupy more than 300 thousand hectares of the country's agriculture land (MARM, 2010; FAOSTAT, 2011). Organic agriculture account for less than 1% percent and integrated production account for less then 16% of total citrus farm land (Igual and Server Izquierdo, 2001; MARM, 2011). The average use of insecticides, that are mainly chlorpyrifos and dicofol, is more than 1 kg of active substances per hectare per year (Muthmann and Nardin, 2007). More than 90% of sprayed insecticides reach a destination other than their target species . Chlorpyrifos and dicofol are toxic compounds. They are toxic particularly to aquatic organisms (Miller, 2004; NIOSHA, 2005a; NIOSHA 2005b). The problem of the conventional pesticides is also that the target pest arthropods, whose are responsible for global 20-50% losses of potential production, became to be relatively quickly resistant to these substances (Thacker, 2002) There is great emphasis on reduction of conventional pesticides application and investment of alternative practices for pest control is highly recommended (FAO/WHO, 2007; OECD, 2008; UNEP 2011).

The loss of biodiversity due to utilization of high amount of chemicals has in the consequences essential impacts on human well being. The loss of biodiversity brings also serious socio-economic and political problems (Altieri and Nicholls,

1994; IUCN, 1994; Moran and Bann, 2000). Artificially maintained agriculture systems are generally monocultures. A number of studies demonstrated that the monocultures are unstable, fragile and transient systems requiring costly external inputs. Ecologists promote that the breakdown of ecosystems can be repaired by restoring the shattered elements of community homeostasis through the addition or enhancement of biodiversity. Traditional, multiple cropping systems are more sustainable and they have richer habitats representation. It has been also detected that the majority of biodiversity is concentrated in the uncultivated marginal lands. Nowadays, a considerable number of studies and projects focus on the function of ecosystems and biodiversity of the agriculture systems (Altieri and Letourneau, 1982; Loomis, D. J. Connor, 1992; Shiva, 1993; Altieri, 1994; Schulze and Mooney, 1993; Altieri, 1999).

Sustainable, sometimes also organic or ecological or conserving agriculture, emphasizes the conservation of natural resources and it benefit the environment through habitat protection, landscape maintenance and the reduction of environmental pollution (Redman, 1992; Rasul and Thapa, 2004). Ecological agriculture takes advantage of pest biological control among the others. It involves the utilization of natural enemies of pests arthropods. Natural enemies can reduce pest population to acceptable levels especially during critical epochs of crops development (Edwards, 1987; Reganold *et al.*, 1990). Despite the fact that sustainable agriculture has many advantages, it occupy only up to 20% of croplands (Altieri, 1999). One of the viable low-impacting options for the pest management is conservation biological control. It is the practice of enhancing natural enemy efficacy through modification of the environment or of existing pesticide practices. It aims at improving the efficacy of natural enemies and can contribute to safer and more effective biological control practices (Jonsson *et al.*, 2008).

Many studies focused on diversity and abundance of natural enemies have been conducted in past decades. However, only small number has been focused on the influence of cover crops on citrus plantations on their biodiversity and abundance. This study contributes to the part of the project which leads Dr. Ing. Rosa Vercher Aznar, member of the Mediterranean institute of agroforestry, in the Polytechnic University of Valencia. The impact of habitat manipulation is discussed in length. There are indications, that the alternative alimentation source, microhabitat

and other services provided by increased plant diversity have positive effects on diversity and abundance of arthropod natural enemies of pest arthropods (Landis *et al.*, 2000) Few studies uttered, that the presence of alfalfa, *Medicago sativa* (L.), within the agroecosystem positively influence the abundance and the diversity of natural enemies (Perdikis *et al.*, 2011)

The aim of this study was to compare the abundance and the diversity of natural enemies, within an ecologic citrus orchard, according to five different cover crop types including alfalfa. There are also some hypothesis, that the abundance and the diversity of natural enemies can be increased if some perennial hedges lie close to the agroecosystem (Wratten *et al.*,1998). The results of this study could contribute to improvement of agriculture techniques in citrus plantations enhancing both farmers and consumers, and it could also contribute to reduction of the pressure on fragile natural ecosystems and biodiversity.

1. Literature review

1.1. Biological control

Biological control (BC) is one of the options for pest management in agriculture (Waage and Mills, 1992). There is number of different definitions of BC in scientific literature (DeBach and Rosen, 1991; Hoy, 1994; van Driesche and Bellows 1996; Barbosa, 1998; Bellows and Fisher, 1999). According to Eilenberg et al. (2001), who recently attempted to unify the definitions relating to the topic, it is the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be. A living organism utilized in this way is called BC agent (Arnold and Anderson, 2002). According to van Driesche and Bellows (1996) BC agent can be parasitoid, predator, pathogen, antagonist, or competitor. Pest is any species, strain or biotype of plant, animal, or pathogenic agent, injurious to plants or plant products (FAO, 1995).

The targets of BC are weeds, plant diseases, some vertebrates but the most important are invertebrates, especially insects and mites which are both arthropods (Van Driesche and Bellows, 1996). BC simply takes advantages of natural BC. Natural BC occurs without human intervention and its estimated value is more than 400 billion US\$ per, year which is considerable higher than the value of 8.5 billion US\$ spent annually on insecticides. Natural enemies of pest arthropods are commonly used biological agents. The pest arthropods with the plant diseases are believed to be the most damaging factors in agriculture. As it has been mentioned above, natural enemy can be parasitoid, predator, pathogen, antagonist, or competitor. This study is focused on arthropod parasitoids and predator, the mostly used agents in BC (Driesche and Bellows, 1996; Driesche, 2002). Hence, the term natural enemy will refer to any arthropod predator or parasitoid of any pest arthropod of any citrus crop within this study. Furthermore, the term pest, will always refer to pest arthropod within this study.

The first BC practice is believed to date to the ancient Egypt where had been cats used for controlling rat populations (Wodzicki, 1973). Until now mentioned, the first BC of pest arthropods by arthropods has been used in China since third century AD (Coulson *et al.*, 1982). The successful release of mynah bird, *Acridotheres tristis*, to Mauritius for control of the red locust, *Nomadacris septemfasciata* in 1762,

is believed to be the begin of modern BC history (Hagen and Franz, 1973). The release of ladybird, *Vedalia cardinalis* (L.) against pest cottony cushion scale, *Icerya purchasi* (Maskell) in California in 1887 is thereafter believed to be the start of control of pest arthropods by arthropods (Doutt, 1958).

The advantages of BC have been broadly discussed. BC is able to avoid or reduce use of them and it doesn't pollute the environment (U.S. Congress, 1995). It is an economical and effective technique in pest control and management in agriculture programs. The cost benefit ratio of BC can reach very high values, highly exceeding the cost benefit ratio of conventional pest management. (Bale, 2008). One of the greatest advantages of BC is that the pest is unable to create resistance against natural enemy (Landis *et al.*, 2000). Other considerable advantage is that the handle with natural enemies is not risky for human health and there is no need of quarantine period within the agroecosystem (Driesche and Bellows, 1996). Many species of natural enemies pose of highly specific host requirements. This feature is often highly desirable due to controllability of the process of control. The host specificity also decrease the possibility of invasiveness and side effects on non-target species (McEvoy, 1996).

High development inputs and long development process without guaranteed positive results are likely to be the greatest disadvantages of BC. The research is usually slow and requires costly expert supervision. These factors, especially the slowness of research and development favour the conventional methods, whose application is very fast and the results are immediate. Another disadvantage is that, the BC is not suitable with conventional pest management methods using pesticides. Firstly, pesticides kill natural enemies directly. In second place, they are killed through feeding on poisoned prey. And in the third place, natural enemies without alternative prey can temporally lose all their source of nutrition and consequently die (Daniel *et al.* 1973; DeBach and Rosen, 1991). BC is broadly used in integrated pest management. Integrated pest management has also number of cited definitions (Stern *et al.*, 1959; FAO, 1967, Smith and van den Bosch, 1967). Kogan (1998) defines integrated pest management as decision support system for the selection and use of pest control tactics, singly or harmoniously co-ordinated into a management strategy, based on cost/benefit analyses that take into account the interest of and impact on producers, society and the environment.

1.1.1. Types of biological control

According to Eilenberg et al. (2001), there are 4 main groups of BC. As a first one can be mentioned classical BC which is likely to be two most known among the others. Nowadays world characterized by international and also by intercontinental movement of great volume of material per day has been and is confronted to high rate migration of species. This migration often results in introduction of undesirable invasive species. Classical BC involves introducing of exotic species of natural enemy, which is often originated to same region as an introduced pest species. This strategy is usually coordinated on large scale governmental level. In the case of successful BC, the benefit cost ratio can reach 1:250 (Driesche and Bellows, 1996; Bale, 2008). Many authors say that establishing of *Vedalia* beetle safe a citrus industry in California. California growers received between 1895 and 1944 exceeded \$3.6 billion, which was as much as \$32 billion in 1991 (Tobey and Wetherell, 1995). The case of Southeastern United States also shows high economical effectiveness of classical BC. The serious pest of rangeland grasses in south Texas Rhodesgrass Mealybug, *Antonina graminis* has been successfully suppressed by realising and establishing of its natural enemies. It has been estimated that between 1974-1978 it resulted in save of 194 millions dollars while the total cost of BC has been estimated to 0,2 million US dollars. (Hokkanen and Lynch, 1995).

The intention of classical BC is to introduce and establish an exotic natural enemy that can control the pest organism for a long period of time or permanently. The steps are choosing the target, acquiring natural enemies, choosing of the natural enemies safe for release, establishing natural enemies in suitable habitats, managing the adoption process and assessing final outcomes (Gurr and Wratten , 2000). When it is successful it takes 10-15 generation of natural enemy to start reduce pest population. Natural enemies once established persist in environment and its population depends on pest population. The introduction has permanent character, it is self-perpetuating. This is the main argument of opponents of classical BC. The impact on the non-target species can be direct, by predation or parasitism or indirect by competing. However, over the past 120 years, more than 5000 introductions of approximately 2000 non-native natural enemies have been made against arthropod pests in 196 countries or islands with remarkably few environmental problems. Thus,

classical BC still provides a relatively safe, and potentially very effective, tactic for control of exotic pests. (Wajnberg, 2001; Bale, 2008).

In many cases, classical BC failed due to unsatisfactory conditions for permanent establishing of biological agent. In these cases there is still possibility to use biological agent in second type of BC which is called augmentation. This technique also involves releasing of natural enemies into the environment, but it has no permanent character. There are two types of augmentation. This concept was first described by DeBach and Hagen (1964). Inoculation involves releasing small numbers of natural enemies at prescribed intervals throughout the pest period, starting when the pest population is very low. Periodic releases of the parasitoid, *Encarsia formosa*, are used to control greenhouse whitefly, and the predaceous mite, *Phytoseiulus persimilis*, is used for control of the two-spotted spider mite especially in Europe (Bale *et al.*, 2008)

Inundation involves releasing large numbers of natural enemies for immediate reduction of a damaging or near-damaging pest population. It is a corrective measure. The expected outcome is immediate pest control. Because of the nature of natural enemy activity, and the cost of purchasing them, this approach using predaceous and parasitic insects is recommended only in certain situations, such as the mass release of the egg parasite *Trichogramma* for controlling the eggs of various types of moths. Inundation BC is used, for instance, to control lepidopteran pests with entomopathogenic viruses and the bacteria *Bacillus thuringiensis* (van Driesche and Bellows, 1996; Bellows and Fisher, 1999).

However, of the three general approaches to insect BC, augmentation is the least sustainable because it requires regular or periodic purchase of products. Nonetheless, in some pest situations it is a highly efficacious, cost effective, and environmentally sound approach to pest management. Augmentation is relatively high cost technique so it is used especially for the treatment of products of high economic potential such as vegetables and ornamental plants. This technique also allows to produce residues-free products which are highly desirable especially in the case of cut flowers and products for human consumption. (Gurr and Wratten, 2000).

Augmentative BC including both inundative and inoculative releasing of natural enemies has a number of ecological limitations. Some of them can be improved (e.g. release timing, release methods, enemy quality, etc.) by researching,

but some of them seems to be technically impossible to alter such (e.g. plant provide a refuge for the pest from the parasitism, etc.) The augmentative BC is not likely to be a remedy for all agriculture production, and is unlikely to replace pesticides on its own in pest management in the near future. However, there has been reviewed considerable number of cases where augmentation *was* effective both in terms of suppression relative to target densities or pesticides, e.g. *Aphytis melinus*-*Aonidiella aurantii* in citrus. (Bale, 2008)

1.1.2. Conservation biological control

Conservation biological control (CBC) is the third type of BC. In literature, it has not received as much attention as other types of biological control. Nevertheless, interest for CBC has increased considerably over the past two decades (Cowgill, *et al.*, 1993; Barbosa, 1998; Bellows and Fisher, 1999; Landis *et al.*, 2000; Jonsson *et al.*, 2008). Many authors define CBC with slight differences, but all of them refer to manipulation of environment and habitat in diverse ways resulting in pest suppression under levels of economical importance. CBC can be divided into two groups according to effect of habitat manipulation on arthropod pest populations. The first one, likely to be minor, consist of manipulation methods resulting in distraction of pest populations off of the planted crop. It is accomplished either by preventing the pests from reaching the crop or by concentrating them in a certain part of the agroecosystem where they can be easily exterminated. This method exploit attracting feature of divers plant species, called trap crop. The attractiveness of trap crops can be also enhanced using specific chemical compound, called attractants, such an insect pheromones (Hokkanen, 1991). It is possible to combine trap cropping and attraction of natural enemies. There is a method of pest management, called push–pull strategy, which is among the others only effective method to control the steam borers, important pest of maize, in eastern Africa. This method involves trap cropping of Napier grass producing high amount of green leaf volatiles, which attract steam borers. The second stage of this strategy involves planting of *Desmodium* spp., which works as a repellent for the pest species and ,as recently discovered, it also increase the activity of natural enemies when intercropped with maize (Khan *et al.*, 2008). This method is known for long time. As early as in the end of the sixties, Stern (1969) described intercropping of alfalfa, *Medicago sativa* (L.) with cotton, *Gossypium hirsutum* (L.) for the control of important mirid bug, *Lygus hesperus*

(Knight). It was observed that the mirid (Heteroptera:Miridae) bug prefers as a host plant alfalfa than cotton, but it migrate within 24 hours to cotton when alfalfa is removed.

Attraction of natural enemies belongs to the second group of CBC, which receive considerably higher attention in literature (Jonsson *et al.*, 2008). CBC of pest arthropods by other arthropods is essentially enhancing of populations and diversity of natural enemies within the agroecosystem through diverse methods of habitat manipulation. In contrast to classical BC, CBC seeks to do better use of existing beneficial insects rather than introducing exotic species. It involves the use of tactics and approaches that involve the manipulation of the environment of natural enemies to enhance their survival and physiological behavioural performance. It results in enhanced effectiveness of pest suppression. CBC of arthropods by arthropods is accomplished through diverse appropriate implementation practices, especially through the creation of infrastructures of non-crop vegetation providing different services enhancing natural enemies abundance and diversity (Barbosa, 1998; Eilenberg *et al.*, 2001; Perdikis *et al.* , 2011). In the early stages of research, CBC of arthropods by arthropods haven't received a lot of confidence, among the other types of BC, due to lack of knowledge and it was considered more as potential rather than realized practice (van den Bosch and Telford, 1964). Since then, a great work on the field of research on habitat manipulation has been done (van Lenteren, 2005).

The increase of diversity and abundance of natural enemies can be accomplished by providing them diverse services: (i) food source; (ii) alternative prey; (iii) shelter habitat; (iii) connecting corridors; (iv) altering current cultural practices (Landis *et al.*, 2000). This services are provided by different cultural strategies. Manipulation of plant species diversity is a method with key effects among the others, because the vast majority of habitats of natural enemies consist of living or death plant material. It is believed, that the increasing plant species richness positively effects the abundance and especially natural enemies species richness (Cowgill *et al.*, 1993; Wratten *et al.*, 1998). The stupendous advantage of CBC, in contrast to classical BC is avaiability for small farmers, because they are familiar with cultural practices. Tremendous advantage over conventional pesticides is that the CBC is completely non-polluting, so the harms on environment and biodiversity are prevented and it is utterly safe for humans. From the point of view of pest

management which is the main objective of CBC the pests are practically unable to become resistant. Advantages over classical BC is that the environment is not exposed to the risks of releasing of exotic species which could affect non-target species. It is highly safe and it exploit primarily indigenous species. Thus the risks of invasive species are negligible. Furthermore, once the effective method is found agroecosystem does not require as much inputs into pest control as in the case of the convectional pest control (Altieri, 1994; van Driesche and Bellows, 1996; Barbosa, 1998). From the economical point of view, CBC is not as effective as successful classical BC, but it reward both landowners and society as a whole by services that are difficulty measurable. Conservation of biodiversity, lower contamination resulted in healthier environment, among the others (Cullen *et al.*, 2008).

Although, CBC has many advantages, it can also generate effects that may be counter-productive to the overall goals of integrated orchard pest management. The enhance of pest densities due to attraction belong to them and is considered as the most serious among the others (Prokopy, 1994). There was also observations that the higher diversity of natural enemies could weakens, or has no affect, on biological control due to competition and mutual predation and parasitism (Straub *et al.*, 2008). In every discipline, there is always some delay between research and adoption of the knowledge and it employment. However, the implementation of knowledge within the field of BC in general difficulty stumbles behind the research with long periods of adoption which often finish with failure. This situation is believed to be caused by both farmer's conservatism and scientist's inaccessibility (Cullen *et al.*, 2008; Griffiths *et al.*, 2008).

1.1.3. Habitat manipulation for natural enemies

Habitat manipulation include both artificial and natural elements adding or altering to favour natural enemies. However, the practices as a providing of artificial nests can have positive effects, the vast majority of researches have been focused on the influence and significance of vascular plants (Jonsson *et al.*, 2008; Hosang *et al.*, 2010). It is believed that the improvement of food sources can results in higher diversity and abundance of them. Gurr and Wratten (1999) adding, that the providing of food sources can be employed for the enhancing of possibilities for the success of classical biological control. The vast majority of arthropod natural enemies are

polyphagous or they change their feed behaviour according to their ontogenetic stage.

Nectar and pollen produced by plants contribute important food sources for many natural enemies. Some predatory Syrphid flies, and some parasitic Hymenoptera, need pollen and nectar for survival during their maturity. Some predaceous beetles and spiders feed on pollen and nectar when there is lack of prey (Leius, 1960, Bakker and Klein, 1992; Cowgill *et al.*, 1993; Taylor and Pfannenstiel, 2009). There was also found strong influence of distance from the nectar source to parasitism rate on aphids by some chalcid wasps (Tylianakis *et al.*, 2004).

Shelter habitats, the refugia, contribute another type of service promoting natural enemies. The general function of shelter habitats is to provide beneficial arthropods with habitats offering suitable biotic and abiotic conditions for overwintering, aestivation and reproduction, and a refuge from the climatic and anthropogenic disturbances. They can be found within or outside of the agroecological systems. In Europe, farmers leave some cluster-forming grasses within the agroecosystems which serve for overwintering of polyphagous beetles. These patches are called “beetle banks” (Barbosa, 1998; Griffiths, 2008).

Altering of current agricultural methods is believed to be one of the most influencing factors among the others. Especially use of pesticides has great impact on densities and diversity of natural enemies. While sprayed, they are directly killed directly, or they feed on infected prey what also lead to their die. It was also documented, that the complete extermination of pest distracts natural enemies and extend their dispersal out of the agroecosystem. Thus, crops are not protected against further outbreaks.

1.1.4. Natural enemies

There is a tremendous number of natural enemies' species in all ecosystems, but especially in tropics, subtropics and temperate zones they are very diverse and abundant. Within BC, the term natural enemy is referred to as parasites, predators and pathogens of any pest arthropod species (DeBach and Rosen, 1991; van Driesche and Bellows, 1996). Natural enemies with good potential tend to have 3 characteristics in common: (i) colonizing ability to allow the enemy to keep tempo with the spatial and temporal disturbances of the habitat; (ii) the ability temporal

persistance in the agroecosystem even if there is a lack of target pest; (iii) opportunistic feeding behaviour to allow the enemy rapidly suppress the pest population. (Ehler and Hall, 1982; Hawkins *et al.*, 1997, Barbosa, 1998). Pathogens are considered as the least important within the field of BC. They have microbiological character, and they are basically studied separately of predators and parasites. Natural enemies with parasitic behaviour are the mostly used in BC followed by predators (DeBach and Rosen, 1991).

1.1.4.1. Natural enemies with parasitic behavioural

Parasites are often smaller than their hosts and develop inside or are attached to the outside of the host's body. Parasites are most often more specific on their prey than predators, which make them quite more exploitable than predators. For pest insects, parasitoids are often the most effective natural enemies. They complete their ontogeny development on a single host (Austin and Dowton, 2000; (Driesche *et al.*, 2008). The vast majority of parasitoid are wasps, order Hymenoptera. There are also some parasitic species within the orders Diptera, Coleoptera and others, but their importance compared to wasps is negligible. The order Hymenoptera is cosmopolitan taxon with more than 115,000 described species, but there are some estimations, that it could be 5-10 times more (Austin and Dowton, 2000). The vast majority of the species importante fo pest management are parasitoid or hyperparasitoids. Parasitoidism is characterized by parasitising of individual only during its larval stage and by killing of host individual (Rana, 2009) while the hyperparasitoid species are characterized by parasiting on already infected individual by another primary parasitoid. However, the life story of the infected individual, for example aphid (Hemiptera: Aphidoidea[Latreille]), is terminated by maturing of either parasitoid or hyperparasitoid (Hoy and Yack, 2009). It is believed take the order hymenoptera pose of the greatist diversity of parasitic species among all insects. There are 40 described families within this taxon. Many of them are broadly used in different BC programes and there are great concerns. of researchers on them. The main superfamilies employed in BC are Ichneumonoidea and Chalcidoidea.

The superfamily Chalcidoidea is one of the most numerous insect taxon. It has about 22,000 known species. However, recent estimates suggest that there may be more than 500,000 species in existence (Gauld and Bolton, 1988). Members of the superfamily Chalcidoidea parasitize on almost all orders of insects. Nevertheless, the

most frequently attacked are the orders Coleoptera, Diptera, Hemiptera, Homoptera and Lepidoptera (DeBach and Rosen, 1991). The superfamily Chalcidoidea includes the families that are the most used in BC. There is ten families which contain considerable number of parasitoid species within the taxon. Many of them are used in BC programs in citrus orchards.

The families Encyrtidae and Aphelinidae are considered as the most frequently used in BC of arthropods (van Driesche and Bellows, 1996). Members of the family Aphelinidae host on eggs of diverse taxa such a scale insects, Coccoidea; greenhouse whiteflies, *Trialeurodes vaporariorum* (Latreille), aphids, Aphidoidea or Psilidae. The members of the genus *Encarsia* (Foerster) also host on the individuals of California red scale (Pina de Montalgrao and Verdú, 2007; Asplanato and Garcia Marí, 2002) or on Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), (Soto *et al.* , 2001). The Species *Coccophagus* (Westwood) host mainly on scale insects. (Compere, 1931). *Aphytis* sp. and *Encarsia* sp. are parasitoids of many groups of arthropods. They host on the taxa Coccoidea, Coleoptera, Diptera, Neuroptera, Orthoptera, Araneae, but also on other species of the order Hymenoptera (van Driesche and Bellows, 1996) The species of the family encyrtidae (Walker) genera are parasitoids of the family Coccidae. They host for example on black scale, *Saissetia oleae* (Olivier), or scale insect ,*Coccus hesperidum* L., (Tena et. al., 2007). Mymaridae (Haliday) They host on taxa Hemiptera, Psocoptera, Coleoptera, Diptera and Orthoptera (van Driesche and Bellows, 1996). The species of the family pteromalidae (Dalman) and eulophidae (Westwood) are very important in BC, particullary in citric orchards. The species of this family host on important pests of citruses. The main host taxa are Mediterranean fruit fly, *Ceratitis capitata* sp.; insect scales *Saissetia* and *Ceroplastes* spp., and *Toxoptera citricidus* sp. (León, 2005). Another superfamily, Ichneumonidae significantly contributes to BC of pest arthropods. The vast majority of species of this superfamily are parasitoids. Members of this superfamily belong either to family Braconidae or Ichneumonidae. Many species of this family are parasitoids of the orders Lepidoptera , Coleoptera, but some of species also host on other members of the order Hymenoptera. Furthermore, some of the species are considered as a hyperparasitoids (García Marí and Ferragut, 2002). The species of the numerous family Braconidae are parasitoids of many orders within the class Insecta. Nevertheless, any of them is considered as a hyperparasitoid

(García Marí and Ferragut, 2002). There are also species, as *Scutellista cyanea* sp. that have predaceous behaviour in larval stages and they became parasitic when mature. They parasite on many pest species, e.g. moths, aphids, scale insects and others (DeBach and Rosen, 1991, Flint and Dreistadt, 1998).

1.1.4.2. Predators

Predators, in contrast to parasitoids, usually feed on great number prey species and they need various or many individuals to feed on for complete their development. Many of them have predaceous behavior during all stages of development. However, vast majority are polyphagous species that can feed on plant foliage, nectar or pollen (DeBach and Rosen, 1991). Many species of natural enemies belong to the order Coleoptera. Lady beetles, family Coccinellidae, are the most common and well known predaceous taxon in BC as a whole. They have great credit of development of BC, due the great success of introduction of Vedalia beetle, *Rodolia cardinalis* (L.) in Florida against cottony cushion scale, *Icerya urchasi* sp. As it safed citrus industry of whole state, it started an epoch of new approach on the potential of BC (Herting and Simmonds, 1971). Pradation is very common within and out of the agroecosystem. There is great number of predaceous taxa but the main imporant and used in BC control are: (i) lacewings, the order neuroptera; (ii) spiders, the order Aracnidae; (iii) syrphid flies; the family Sirphidae; (iv) dragon flies, the order Odonata. This groups are possibly the most commons predators as a predator fauna in citruses (DeBach and Rosen, 1991; Alvis Dávila and García Marí, 2006).

The order neuroptera is believed to be very inportant part of BC. In spain, the families Coniopterygidae, Chrysopidae are the most common. There are 3 very common species within the family Coniopterygidae. *Coniopteryx* spp., *Conwentzia psociformis* (Curtis) and *Semidalis aleyrodiformis* (Stephens).

1.1.5. Cover crops and natural enemies in citrus plantations

Cover crops have been already using in the past decades in agriculture and livestock production as fodder, anti-erosion agents and they have been also utilizing to improve soil fertility and it's physic-chemical properties, and food production (Lal *et al.*, 1991; Hermawan and Bomke, 1997; Snapp *et al.*, 2005). There had been emphasizes for utilization of cover crops for weed control as a living mulches and/or for establish the competition between cover crop and weed. Emphasis are recently

focused on utilization of cover crops for conservation biological control. Nevertheless, there are also negative influences as competition for water and nutrients and attraction and habitat provision to the pest (Spitters and Van den Bergh, 1992; Teasdale, 1996; Barbosa, 1998; Coll, 1998 and 2009). Studies of individual cover crop species can estimate their promotion of pest or natural enemy species. There are four basic favouring mechanisms promoting natural enemies. For example *Vaccinium* spp.; dogwood, *Cornus* spp.; holly, *Ilex* spp.; *Fraxinus* spp.; hawthorn, *Crataegus* spp.; and nettles, *Urtica* spp. are considered to support alternative hosts and prey for parasitoids and predators. Chrysopids, coccinellids, syrphids and parasitoids have been shown to use extra-field nectar sources, alternative food source, and spread into surrounding crops. Extrafloral nectar which allures above mentioned natural enemies produce for example *Vicia faba* L., *Gossypium hirsutum* L. and *Brassica oleracea* L. It has been detected that for example *Medicago sativa* L. provide suitable shelter and microclimate for some coccinellids with adequate crop management. Some *Rosae* spp. promote natural enemies by over wintering and nest habitat (Pickett and Bugg, 1998; Landis *et al.*, 2000; Bianchi *et al.*, 2006).

Citric orchards are traditionally maintained without cover crops except winters especially for management facilities (Soler Aznar and Soler Fayos, 2006). However the orchards without cover crops have low water infiltration ratio what increase the erosion ratio and produce sediments run-off (Wu *et al.*, 2003). Pons Mas *et al.* (2000) also demonstrated beneficial properties of some species for competition with the weeds and there have been made some experiments which confirmed beneficial soil improving prosperities of some species of cover crops especially some leguminous (Ingels *et al.*, 1994; Sarrantonio and Gallandt, 2003). Perdakis *et al.* (2011) observed high densities of *Geocoris* spp. in intercropped alfalfa with cotton and migration toward crop. Landis *et al.* (2010) also observed considerable increase of abundance of parasitic Hymenoptera species, when alfalfa was present.

With deeper knowledge about habitats which promote natural enemies of main citric pests could be orchards composed in the way leading to sustainable yields, energy conservation, and less dependence on external inputs (Altieri, 1999; Bottrell and Barbosa, 1998). There have been made some experiments that demonstrated that cover crops can be also incorporated as supplementary sources of natural enemies of citrus arthropod pests (Liang and Huang, 1994; Grafton-Cardwell,

1999) but the science of habitat management is still in its infancy (Landis *et al.*, 2000) and there is a clear need for large-scale, well-replicated studies in this field (Bugg, 1991).

2. Objectives

The aim of this study was to compare the biodiversity and the abundance of natural enemies of pest arthropods in ecologic citrus orchard according to different cover crop types and different distances from hedges. The ambition of this study was to analyze and to evaluate obtained dataset. Thus, it was possible to confirm or negate the hypothesis, that there are certain aspects that increase the diversity and the abundance of natural enemies of pest arthropods within citrus orchards. Expected results of this study should contribute to entire understanding of the influence of the plant species composition on the diversity and the abundance of natural enemies in citrus plantations. Hence, the results of this study could contribute to improvement of agriculture techniques in citrus orchards enhancing both farmers and consumers, and it could also contribute to decrease the pressure on fragile natural ecosystems and biodiversity. The questions attempted to be answered are. Does the type of cover crop influence the abundance and taxa richness of natural enemies within the citrus orchard? If yes, which plant species influence it positively? Do the nearby hedges influence the abundance and taxa richness of natural enemies? And if so, how?

Specific objectives of this study are:

- (i) create a catalogue of natural enemies associated to citrus orchard and to selected cover crops;
- (ii) compare the proportions of natural enemies within the crop layer and tree layer;
- (iii) compare the abundance of natural enemies between the tree and cover crop layers;
- (iv) compare the taxa richness and abundance of natural enemies according to cover crop type for particular layers and for whole plots
- (v) compare the diversity of natural enemies according to type of cover crop and distance from the hedges, using diversity indices
- (vi) compare similarities of species composition between plots of different cover crops

3. Materials and methods

3.1. Study site

The were collected at the ecologically grown citrus orchard called Mandarinas Ecologicas Ricarde, located 5 km south east of the centre of the town Alzira, region Ribera Alta in the province of Valencia, Spain (+39° 8' 7.27", -0° 24' 24.42") (Figure 1.). The orchard is situated in the valley of La Vall de la Casella close to the natural park “La valle de la Murta”. The province of Valencia belong to the regions of high production of citruses and other subtropical fruits and vegetables.



Figure 1. The location of plots, where arthropods were captured for the dataset
Source: maps.google.com

The study site lies about 40 km south of the city Valencia and its climate is considered as typically Mediterranean highly influenced by Mediterranean sea. The average annual temperature is 17,8°C, where the average temperature of the daily maxims is 22,3°C and the average temperature of the daily minims is 13,4°C. In general, the temperatures not change considerably during year nor during the day and night. The temperatures below 0°C are exceptional and occur rarely few times per

year for very short time. The precipitations are distributed mainly in colder months and their annual average range about 450 mm. The vast majority of their volume shower within a few days of the whole year (only 44 days in whole year exceed precipitations of more than 1mm/day). The humidity is very stable about 65% whole year and the weather is highly dependent on the direction of wind currents. The annual average of sunny hours is 2660 per year (Instituto Nacional de Meteorología, 2004). From the geological point of view, the site lies on the Valencia tough which has been recently formed by Oligocene to early Miocene rifting phase that was followed by a period of post-rift subsidence and a late Miocene-Pleistocene phase of renewed crustal extension and magmatism; and it resulted in highly attenuated continental crust (Banda and Santanachb,1992; Capote *et al.*, 2002). The soil type in the region range from Fluvents to Xerals, but the citrus plantation predominate on Fluvents, Orthents, Arents, Cambisols with high content of calcium carbonate. This lime-rich soils are moderately alkaline with pH 7-9 (Ayala Carcedo, 1988; Ingelmo Sánchez, F. 1990) From the production point of view, the study site belong to to the group B, with moderate limitations, moderate risk of erosion and capacity for moderately intensive agriculture (Antoliín Tomás *et al.*, 1998). Typically Mediterranean Mattoral shrubland predominate in this region. The most abundant species on large scale are *Quercus Ilex L.*, *Quercus coccifera L.*, *Thymus L.*, *Lavandula L* and other perennials (Barbero *et al.*, 1992)

The orchard possess an area of 3.96 hectares. The production consist of growing clementine mandarins, *Citrus clementina* (hort. ex Tanaka). The trees are same aged about 20 years old. There are cultivated 3 varieties of clementine mandarins: (i) Beatriz; (ii) Orogrande; (iii) Nules. The distribution of particular varieties can be seen in the plan, Figure. . The owner of the orchard is farmer, but also researcher of Valencian institute of agrarian researches. Thus he carries on ecological based technologies of crop management, for experimental purposes. Secondary effect is production of considerably volume of certificated “ecological” fruits of high market value.

3.2. Data collection

There was great emphasis on collecting the most possible number of arthropods and the most possible number of taxa in different layers within the orchard. There exist a number of methods for sampling arthropods (Southwood et al. 1982; Hradezky and Kromp, 1997; Paoletti *et al.*, 1997). They differ in spatial and temporal patterns, the equipment used for the collection of samples, corresponding to the purposes of a studied topic. According to expected characteristics of demanded arthropods, has been chosen the method developed by Maudsley *et al.* (1997). There have been selected 9 plots of 15x20 metres within the orchard. It consisted of four pairs of plots, each with the same cover crop differing in distance from the hedge and one plot without variant (Figure). The distance of the plots based closer to the hedge was less than 5-15 meters from the hedge (labelled as variants 1) and the distance of the plots based farther from the hedge was 30-40 meters from the hedge (labelled as variants 2). These are the sampled plots:

(i) F+A1 and F+A2 = Two plots with cover crop consisted mainly of *Festuca* (L.), but there were also considerably patches of *Lolium rigidum* (Gaudin), *Medicago sativa* (L.), *Medicago truncatula* (Gaertn.), *Dactylis glomerata* (L.) and *Melilotus officinalis* (L.). Both plots were re-sown in 2010. Thus, the individuals of mentioned species occupied whole plot with only tiny patches of other plant species;

(ii) A1 and A2 = Two plots of alfalfa, *Medicago sativa* (L.) Both plots were sowed in 2006 and so alfalfa was not completely dominant. It comprised 50-60% of ground cover depending on season and it is partly accompanied by *Cynodon dactylon* (L.) Pers., plus few small patches (about 10x10) of *Rumex* (L.), amaranths, *Amaranthus* (L.). (*A. retroflexus* [L.], *A. hybridus* [L.] , *A. blitoides* [L.]) and other grasses, Poaceae (Barnhart) and weeds;

(iii) F1 and F2 = Two plots of *Festuca arundinacea* (Schreb.). This species comprised 60-70% of ground cover. The rest of ground cover consisted of equally abundant species *Trifolium subterraneum* (L.), *Trifolium michelianum* (Savi), *Trifolium resupinatum* L., *Medicago truncatula* (Gaertn.) and *Medicago sativa* (L.);

(iv) S1 and S2 = Two plots with diverse spontaneously grown species, particularly grasses. The composition has been changing according to the season. Nevertheless, about 50% of ground cover stably consisted of *Cynodon* (Rich.), *Bromus* (Scop.), *Amaranthus* (L.), *Sonchus* (L.), *Chenopodium* sp., *Senecio* (L.) and *Calendula* (L.);

(v) F/F+A = The plot sowed in 2009. The ground cover

consisted of *Festuca* (L.), *Lolium rigidum* (Gaudin), *Medicago sativa* (L.), *Medicago truncatula* (Gaertn.), *Dactylis glomerata* (L.) *Melilotus officinalis* (L.) and *Festuca arundinacea* (Schreb.) in equally abundances.

The hedge consisted of the typically and broadly distributed species *Pistacia lentiscus* (L.), *Crataegus monogyna* (Jacq.), *Rhamnus alaternus* (L.), *Pistacia terebinthus* (L.), *Cupressus sempervirens* (L.), *Punica granatum* (L.) and *Ailanthus altissima* (L.).

The plots A1, A2, S1 and S2 were based within the orchard where were cultivated mandarins of the variety Orogrande and the plots F1, F2, F+A1, F+A2 F/F+A and F/F+A2 lied where are planted mandarins of the variety Beatriz (Figure).

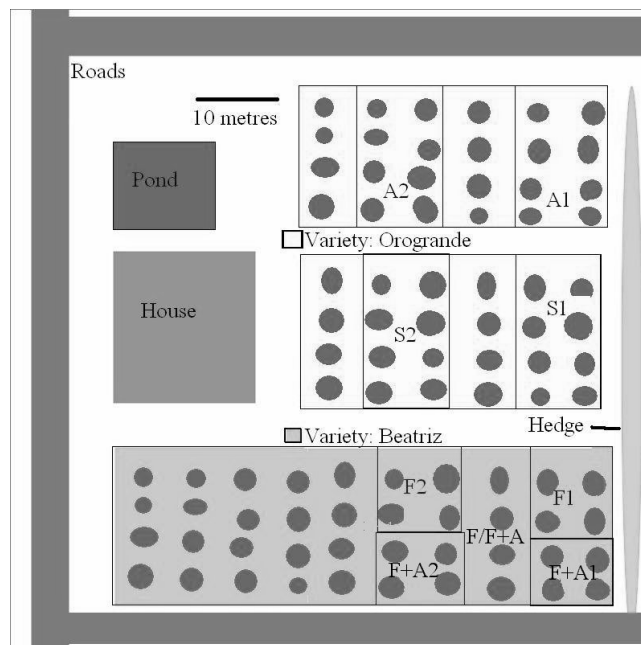


Figure 2. Orientation plan of location of sampled plots with different varieties of *C. Clementine* (L.)

Aspiration (Maudsley *et al.* 1997) was selected as the most realizable and the most available among the others. It is an appropriate method for collecting quantitative data of terrestrial invertebrate communities of free-living adults (Southwood and Henderson, 2000). Sample collection was performed with a vacuum cleaner (gas-engine) brand Komatsu Zenoah Co. HBZ2601 model with a displacement of 25.4 cm³. The vacuum cleaner was adapted on the suction of a plastic cylinder of 30 cm in diameter and 80 cm high. The fishnet stocking is stretched over the connecting part of the plastic cylinder. It serve to catch all the sucked material within it. Each sample is obtained during 2 minutes long sucking of

chosen plot layer and consequential as fast as possible fishnet stocking. Each of the plots were sampled twice per sampling day. One of the samples was taken from the tree layer (more than 1,5 metres above the ground) and the second one was taken from the cover crop layer (0-1 m above the ground). The samples (fishnet stockings) were consequentially putted into the plastic bag and transferred to the laboratory. All the plots were sampled 18 times during approximately one year. The days of sampling were distributed in equally intervals with exceptions. The sampling days were chosen according to the current weather. Moderately cloudy days without rain, considerable wind and other negatively influencing factors were preferred. The sampling always proceeded between 8:00 and 10:00 AM on: (i) 14.7.2009; (ii) 6.8.2009; (iii) 3.9.2009; (iv) 22.9.2009; (v) 8.10.2009; (vi) 29.10.2009; (vii) 24.11.2009; (viii) 29.12.2009; (ix) 29.1.2010; (x) 26.02.2010; (xi) 15.4.2010; (xii) 20.5.2010; (xiii) 14.06.2010; (xiv) 19.7.2010; (xv) 9.8.2010; (xvi) 9.9.2010; (xvii) 27.9.2010; (xviii) 21.10.2010.

In the laboratory, based in the Polytechnic University of Valencia in the department of Agroforestry, the samples were putted into freezers (-20 degrees Celsius) for a minimum time of 24 hours to bring to death all the living arthropods and also to store them. Actual laboratory work started after removal of the samples outside of the freezers and after their drying. Primarily, all impurities (leaves, branches, soil, etc) were removed from the samples, to facilitate the observation and separation of the arthropods. It was done by emptying fishnet stocking on large clear paper and consequential manual replacing of the impurities. Obtained residues, arthropods with impurities too tiny to replace, were separately putted into Petri dishes and isolated by parafilm. Each Petri dish was labelled by sticker with relevant date, plot and vegetation layer. Cleaning was followed by identification. Binocular light microscopes, with magnification 40x-640x, were used for substantial identification of arthropod taxa. All the arthropods were identified to the lowest taxonomic level possible.

The identification has been done with the supervision of Dr. Ing. Rosa Vercher Aznar. There were also few authorities, scientists of the University of Valencia and the Valencian institute of agrarian researches, asked for the help with the identification of problematic taxa. However, the vast majority of the arthropods were identified using collections of the Polytechnic university of Valencia and a

number of identification keys (Killington, 1936; Killington, 1937; Pritchard Earl, 1953; Gómez Menor, 1956; Carayon, 1972; Péricart, 1972; Plaza Infante, 1977; Aspöck et al., 1980a; Aspöck et al., 1980a; Stubbs and Falk, 1983; Plaza Infante, 1986; Raimundo Cardoso and Alves Gomes, 1986; Brooks and Barnard, 1990; Goulet and Huber, 1993; Plant, 1997; Stubbs and Falk, 2003). The primary data have been written for the further elaboration on the clear papers.

3.3. Data evaluation

All the data from the field sheet were passed into the spread sheet Microsoft Excel. It was decided that the data from the different dates will be fused and the data evaluation will be done with absolute amounts of individuals. Firstly, all identified arthropods were catalogued. The table of total amount of arthropod taxa was made. All the individuals from all plots have been summarized in table according to their taxa and the layer where they were captured. This table was simplified to the level of orders, providing a simple view of the abundance of each order. Each taxa was labelled according to its ecological function. From the point of view of farmer, there were five possible ecological functions within the agroecosystem: (i) predator; (ii) parasitoid; (iii) herbivore; (iv) detritivore (v) unspecified. This allowed us to compare the proportions and amounts between differently behaving groups of arthropods. This was able to do for all individuals together and also for individuals of particular layers. The comparison of proportions and total amounts of particular taxa has been also done within the group of predators and parasitoids. All these comparisons were expressed in the graphs.

All the natural enemies were summarized for each sample and also for pairs of samples originated from the same plot. This allowed us to express four factors of biodiversity of particular plots, particular layers, and fused plots covered with the same type of cover crop: (i) total species richness expressed by the total number of taxa per sample/cover crop; (ii) absolute abundance of natural enemies expressed by their absolute number (iii); diversity of each sample/cover crop using commonly employed Shannon's and Weaver's diversity indices; (iv) similarities between the particular samples and between particular cover crops using Sorensen similarity index and Jaccard similarity index.

There was need for use some species diversity measures to express heterogeneity of species for different plots and layers. Simpson's reciprocal index and Shannon's index are broadly used indices of biodiversity. Shannon's index, is believed to emphasize the richness of species, whereas Simpson's index, is believed to emphasize the evenness. Simpson's index is a measure that accounts for both richness and proportion of each species within the area. The values of Shannon's index usually varies between 1,5 and 3,5 and rarely exceeds value of 4,5 or more. (Peet, 1975; Burgio, 1999; Krebs, 1999; Nagendra, 2002). Shannon's entropy H is defined as

$$H' = - \sum_{i=1}^R p_i \log p_i$$

where H' = Index of species diversity

R = Number of species

p_i = Proportion of total sample belonging to i th species

Simpson index is defined as

$$D = \sum_{i=1}^R p_i^2$$

and related Simpson's reciprocal index is defined as

$$1 / D$$

Broadly used coefficient of Sørensen and coefficient of Jaccard were chosen as a good method for similarity assessment between different plots (Krebs, 1999). If the value of coefficients is equal to 1, that means that all of the species are found in both ecosystems and they are identical. If the number equals 0, there is no similarity. The closer this value is to the number one the more similar. (Sørensen, 1957; Wolda, 1981).

Sørensen's coefficient is defined as

$$S_S = \frac{2a}{2a + b + c}$$

where S_S = Jaccard's similarity coefficient

a = Number of species common for both samples

b = Number of species present in sample A but not in sample B

c = Number of species present in sample B but not in sample A

Coefficient of Jaccard is expressed as

$$S_J = \frac{a}{a + b + c}$$

where S_J = Jaccard's similarity coefficient

a = Number of species common for both samples

b = Number of species present in sample A but not in sample B

c = Number of species present in sample B but not in sample A

4. Results

4.1. Catalogue of species

The total number of all captured arthropods reached 47624 of individuals belonging to different taxa of arthropods. These include individuals captured in both tree layers and cover crops layers. The total number of captured arthropods was 9,642 in all tree layers and 37,982 in cover crop layers (Annex 1). The most abundant taxa in tree layers were Homoptera, Hymenoptera, Coccidae, Psocoptera and Aranea with 27.7%, 25.3%, 17.4%, 6.8% and 6.2% of all individuals respectively (Table 1). The most abundant taxa in cover crop layers were Homoptera, Hymenoptera, Heteroptera, Acari and Collembola with 52.8%, 17.2%, 12.8%, 6.0%, and 4.4% of all individuals respectively. The most abundant taxa in total were Homoptera, Hymenoptera, Heteroptera, Acari and Coccidae with 47.7%, 18.8%, 10.6%, 5.4% and 3.7% of all individuals respectively. The captured arthropods can be grouped depending on their ecological function. The groups are predators, parasitoids with hyperparasitoids, detritivores, herbivores, and the group unspecified which is group of arthropods with diverse ecological functions. The individuals of the orders Isopoda, Odonata, Ephemeroptera, Dermaptera and Mantodea were captured in inconsiderably numbers. There were captured only 20 individuals of these orders together. Hence, they can be considered as very marginal group.

The most numerous groups in total were herbivores, parasitoids with hyperparasitoids and predators with 72.36%, 14.43% and 9.73% respectively (Figure 3). The most numerous groups in tree layers were herbivores, predators and parasitoids with hyperparasitoids represented by 53.68%, 23.51% and 14% respectively (Figure 4). The most numerous group in tree layers were herbivores, parasitoids with hyperparasitoids and predators with 77.1%, 14.54% and 6.23% respectively (Figure 5).

Table 1. Total numbers of identified arthropods according their taxonomic affiliation and vegetation layer where were captured

Taxon	Number of individuals			%	
	Tree	Cover Crop	Total	Tree	Cover Crop
Homoptera	2,673	20,052	22,725	27.7	52.8
Hymenoptera	2,439	6,536	8,975	25.3	17.2
Heteroptera	216	4,844	5,060	2.2	12.8
Acari	329	2,266	2,595	3.4	6.0
Collembola	3	1,655	1,658	0.0	4.4
Thysanoptera	87	705	792	0.9	1.9
Aranea	597	535	1,132	6.2	1.4
Diptera	263	531	794	2.7	1.4
Coleoptera	296	304	600	3.1	0.8
Orthoptera	29	224	253	0.3	0.6
Lepidoptera	219	130	349	2.3	0.3
Coccidae	1,677	92	1,769	17.4	0.2
Psocoptera	657	80	737	6.8	0.2
Neuroptera	147	18	165	1.5	0.0
Isopoda	0	6	6	0.0	0.0
Odonata	0	3	3	0.0	0.0
Ephemeroptera	7	1	8	0.1	0.0
Dermatoptera	2	0	2	0.0	0.0
Mantodea	1	0	1	0.0	0.0

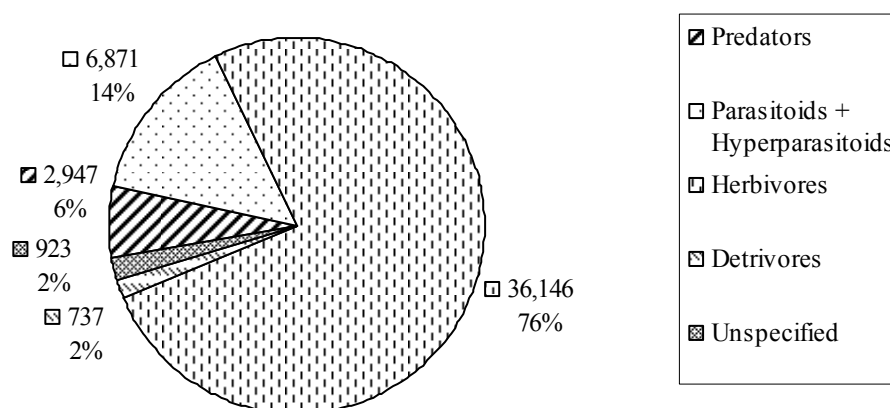


Figure 3. Proportions of the total numbers of particular groups of arthropods according to their ecological function within both tree and tree layer.

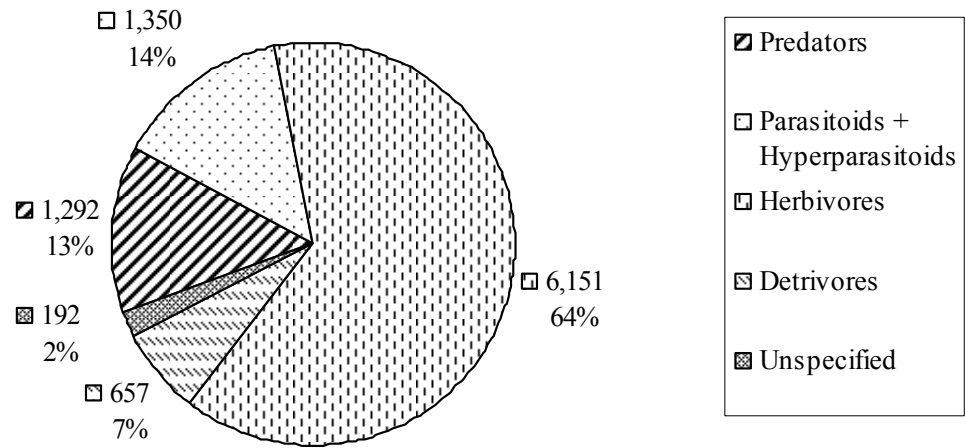


Figure 4. Proportions of the particular groups of arthropods according to their ecological function within tree layer

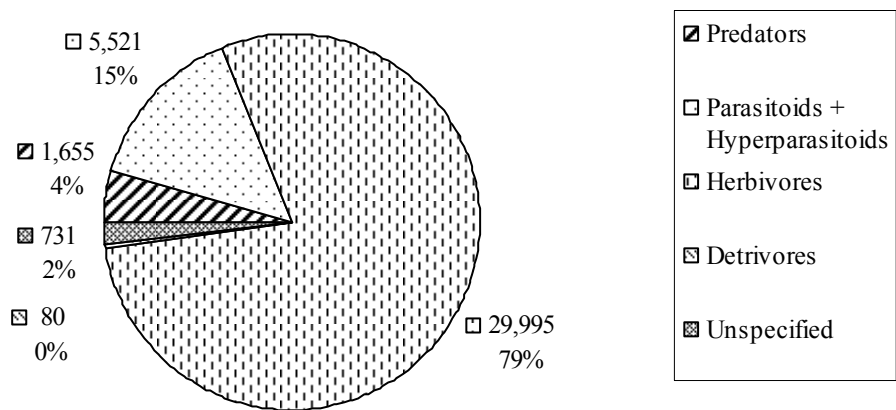


Figure 5. Proportions of the particular groups of arthropods according to their ecological function within cover crop layer

4.1.1. Parasitoids

The individuals of this group were the second most numerous of all captured arthropods. All the individuals identified as taxa with parasitic behaviour belonged to the order Hymenoptera.

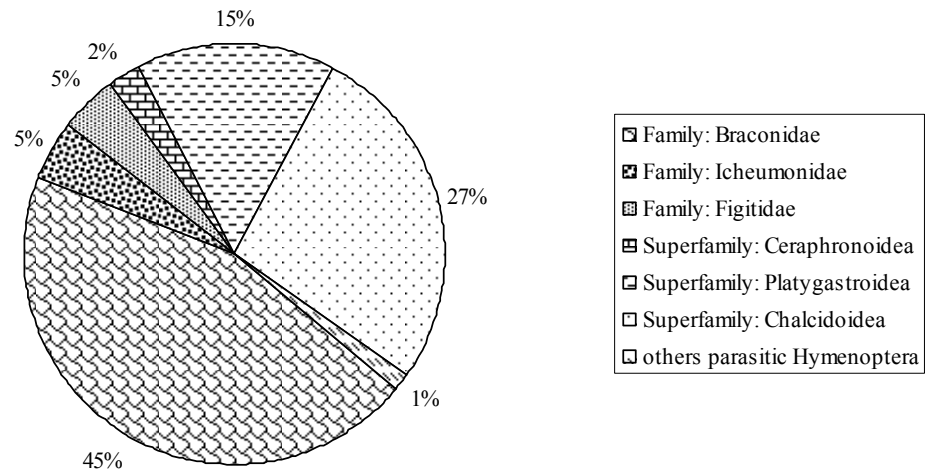


Figure 6. Proportions of the amounts of particular taxa of parasitic natural enemies of the order Hymenoptera within both tree and cover crop layer

The superfamilies Chalcidoidea, Platygastroidea, and the family Braconidae were the most numerous taxa captured in the study (Figure 6). Nevertheless, the superfamily Ceraphronidae, and the families Ichneumonidae and Figitidae can be also considered as an important groups according to their noticeable abundances. There were identified only 2 individuals as members of the family Chrysididae. The individuals of others taxa of hymenoptera comprised insignificant proportion of all parasitic individuals captured.

There were identified 1855 individuals as a members of the superfamily Chalcidoidea. Within the superfamily there were identified some important families. There were 415 individuals identified as members of the family Aphelinidae. Twenty of them was identified as a members of the genus. The rest of individuals was identified only on the level of family but there were some indications that a considerable number of them belonged to the genus *Aphytis* and *Encarsia* (Foerster). Another identified taxon was the family Encyrtidae. There were 162 individuals of this taxon captured in the study. The most abundant group identified belonged to the genera *Methapycus* (Mercet) (66 individuals). Another identified group was the family Mymaridae. There were 267 individuals identified as members of the family.

Another identified family with significant abundance was the family Pteromalidae (Dalman). There were 428 individuals identified as members of this family. Eulophidae, another important group of the order Hymenoptera was also significantly abundant. There were 434 individuals of this taxa captured in the study. Amount of 89 of them were identified as a *Citrostichus phyllocnistoides* (Narayanan). In this study, there were also identified individuals of other families of the superfamily Chalcididea, Trichogrammatidae spp., Chalcididae (Latreille), but their abundance was considerably small as only 17 individuals in total were captured. The amount of remaining 132 individual was identified only on the level of superfamily.

The most abundant taxa was the superfamily Ichneumonoidea. There were 3398 individuals of this superfamily captured in the study. 326 individuals of the superfamily Ichneumonoidea belonged to the family Ichneumonidae and they remained identified on the level of superfamily. The rest of individuals of the superfamily Ichneumonoidea (3072 individuals) belonged to the family Braconidae. The most abundant taxa among this family was the subfamily Opiinae with 1634 individuals. The second most numerous was the subfamily Aphidiinae with 760 individuals captured. The third most numerous group captured were the members of the subfamily Alysiinae which are all parasitoids of flies (Muscomorpha:Diptera) (Trostle *et al.*, 1999). Another numerous group was the subfamily Microgastrinae with 136. The last noticeable taxon was also the subfamily Agathidinae with 65 individuals.

The superfamily Ceraphronidea comprised small fraction of the identified parasitic natural enemies. There were 166 individuals identified as a members of this superfamily. Within this superfamily, there were 34 individuals identified as members of the family Megaspilidae and 85 individual were recognized as members of the family Ceraphronidae. Considerable proportion comprised the individuals identified as members of the superfamily Platygastroidea. There were 1,042 individuals identified as members of this superfamily, which was significant number within the order Himenoptera. Marginal groups comprised the family Figitidae and the superfamily Vespoidea with 311 and 54 individuals respectively. Considerable amount of 472 individuals remained identified on the level of order.

4.1.2. Predators

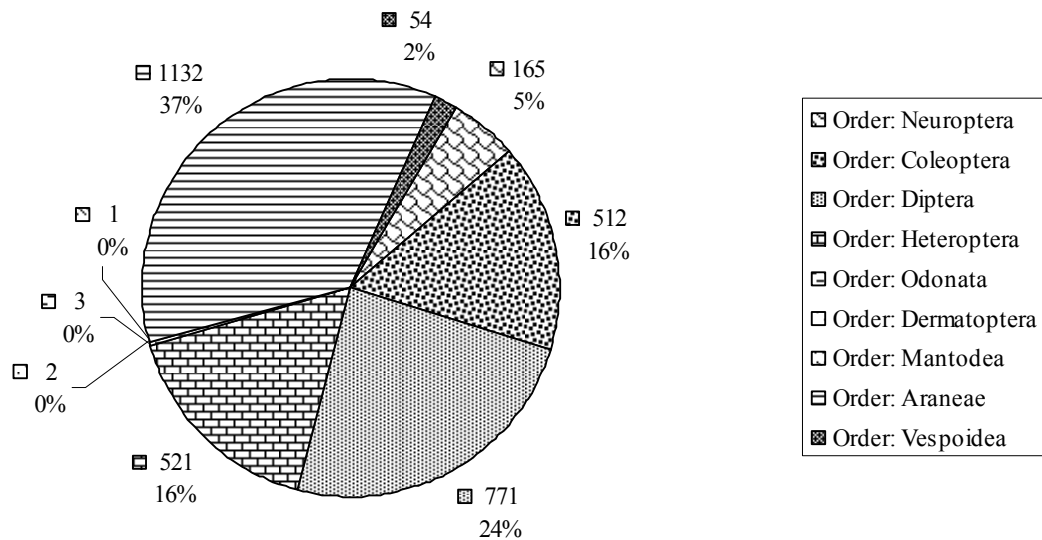


Figure 7. Proportions of the amounts of particular taxa of predaceous natural enemies within both tree and cover crop layer

The orders Araneae, Diptera, Coleoptera and Heteroptera were the most abundant orders of predaceous species identified in this study (Figure 7). The orders Odonata, Mantodea and Dermaptera comprised only marginal proportion of total number of all predators.

There were identified 165 individuals as the members of the order Neuroptera. Within the order Neuroptera, the family Coniopterygidae comprised the most abundant family. There were distinguished 3 species within the family Coniopterygidae. *Coniopteryx* spp., *Conwentzia psociformis* (Curtis) and *Semidalis aleyrodiformis* (Stephens) were represented by 24, 19 and 62 individuals respectively. Chrysopidae comprised the second most abundant family of the order Neuroptera. There were identified two species within the Green lacewings (Chrysopidae). There were captured 55 individuals of Common Green Lacewing, *Chrysoperla carnea* (Stephens), and only 3 individuals of *Chrysoperla septempunctata* (L.). There was identified only one individual as a member of the family Hemerobiidae.

In this study, there were captured 512 individuals of predaceous beetles, the order Coleoptera. There were identified 38 individuals as members of the family

Staphylinidae in this study. Although, there were identified more families, the most abundant and the most important for the purposes of this study were the members of the family Coccinelidae. There were 258 individuals identified as members of this taxon. There were identified 9 taxa of the family Coccinelidae, but only three of them were significantly abundant. The most abundant species was *Scymnus subvillosus* (Goeze) with 180 individuals and the species of the same subfamily *Scymnus interruptus* (Goeze) with 27 individuals captured.

Within this study, there were identified 771 individuals as predaceous species of the order diptera. There were identified 323 individuals as members of the family Cecidomyiidae and 49 individuals as members of the family Syrphidae. . There were also captured individuals of the genus *Platypalpus* with amount of 89 individuals. Another species of the order diptera, *Thaumatomyia notata* (Meigen) was captured in high abundances of 310 individuals. There were also identified 3,2 and 1 individuals of the orders Odonata, Dermaptera and Mantodea. The order Araneae comprised great part of all predaceous natural enemies with the amount of 1132 individuals.

The predaceous species of the order heteroptera comprised also considerable proportion of predaceous natural enemies with 521 individuals. Within this order, there were identified 3 families which have predaceous behaviour. There were identified 179 individuals as members of the family Nabidae and all of them except one were identified as the species *Nabis Pseudoferus* (Remane). There were identified 165 individuals as a members of the genus *Dyciphus* and 35 individuals as members of the genus *Pilophorus* within the family Miridae.

There were identified 97 individuals as members of the family Anthocoridae. Furthermore, the vast majority (72 individuals) of them were identified as a members of the species *Orius spp.* There were identified 45 individuals as members of the genus *Geocoris* (Fallén) within the family Lygaeidae (Schilling).

4.2. Abundances of natural enemies in different layers

In this study, the vegetation layer played important role in the terms of proportions and abundances of natural enemies. The taxa of the natural enemies that were captured in considerable amounts of individuals, allowed to compare the abundances of them between the tree and cover crop layers. There were taxa that

were considerably more abundant in the tree samples or in the cover crop samples, but there were also species that occurred in both layers in similar amounts.

4.2.1. Parasitoids

There were different abundances of particular groups of parasitic Hymenoptera obtained from different layers. There was one significantly more numerous taxon than the others within the group of parasitoids in the tree layer. The superfamily Chalcidoidea leaded this group with 810 individuals, continued by the superfamily Platygastroidea and the family Braconidae with 213 and 199 individuals respectively (Figure 8).

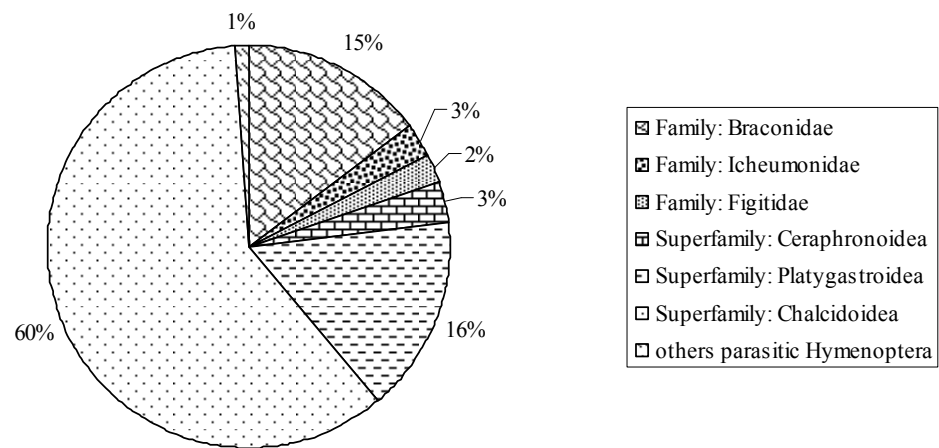


Figure 8. Proportions of the amounts of particular taxa of parasitic natural enemies of the order Hymenoptera within the tree layer

The situation within the parasitoids in cover crop layer was notably different. The most numerous taxon was the family Braconidae with 2873 individuals. The second most numerous was the superfamily Chalcidoidea with 1045 individuals closely followed by the superfamily Platygastroidea with 829 individuals (Figure 9).

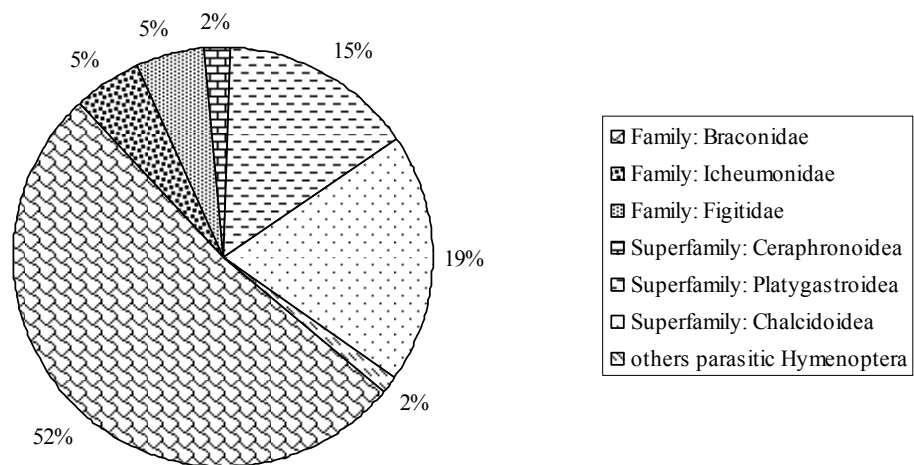


Figure 9. Proportions of the amounts of particular taxa of parasitic natural enemies of the order Hymenoptera within the cover crop layer

All the parasitic taxa which were captured in considerable amounts were also more abundant in cover crop layer as it is seen in the (Figure 10).

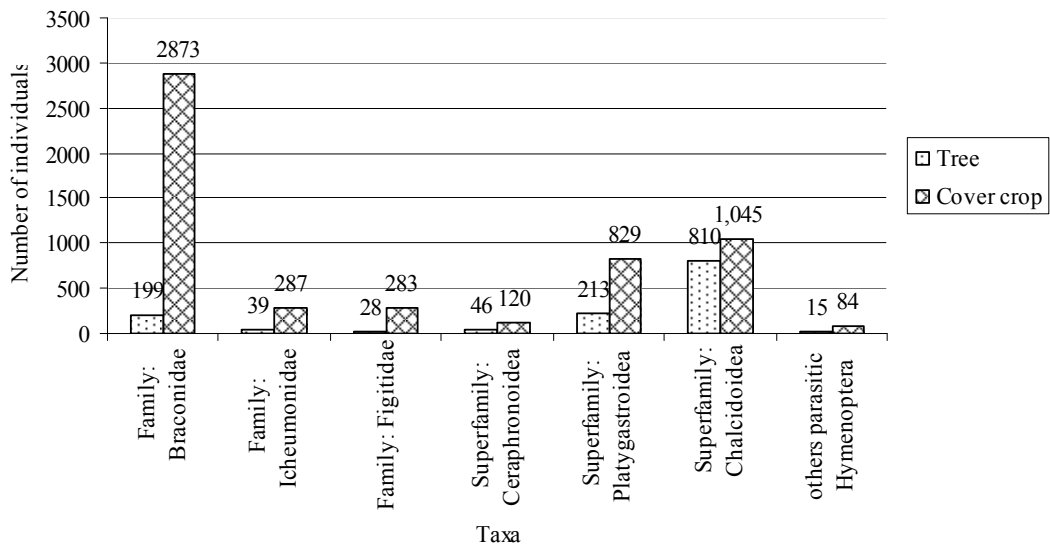


Figure 10. Distribution of the most abundant taxa of parasitic natural enemies according to vegetation layer

The same situation occurred when where compared the amounts of individuals of different taxa within the superfamily Ichneumonoidea. Some of taxa were present almost exclusively in cover crop layer (Figure 11).

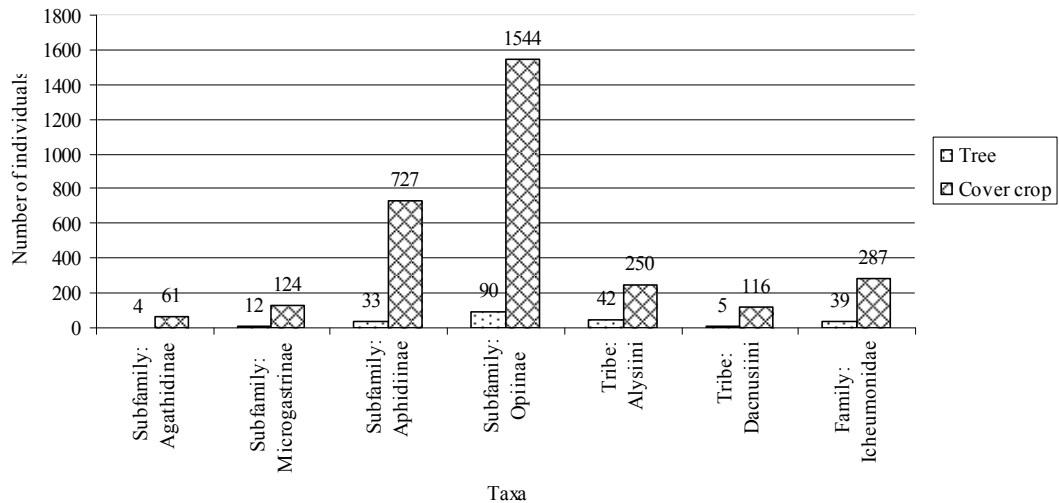


Figure 11. Distribution of the most abundant taxa of the superfamily Ichneumonidae according to vegetation layer

There were observed notable differences between different taxa of the superfamily Chalcidoidea (Figure 12.) Some of the taxa were captured in considerable higher amount of individuals in tree layer (the family Aphelinidae, *Citrostichus phyllocnistoides* [Narayanan], *Microteris nietneri* sp. and the genus *Metaphycus* [Mercet]). There were also taxa that were more numerous in cover crop layer (others Eulophidae, the family Pteromalidae and Mymaridae, the rest of Chalcidoidea which were not identified). The last group consist taxa that were similarly abundant in both layers.

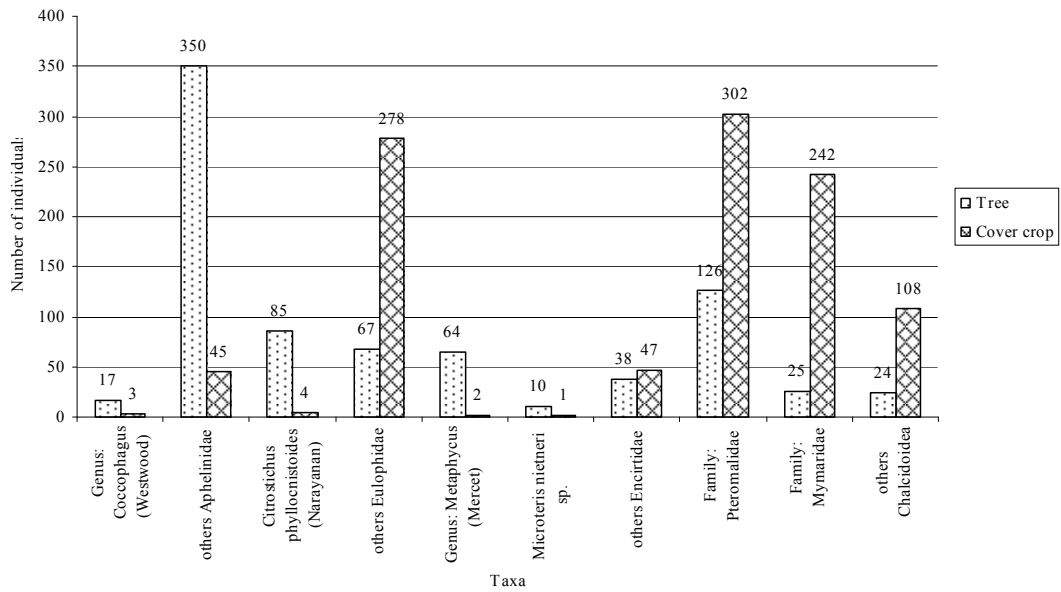


Figure 12. Distribution of the most abundant taxa of the superfamily Chalcidoidea according to vegetation layer

Considerable higher amount of individuals of the superfamily Proctorupoidea was captured in cover crop layer than in tree layer.

4.2.2. Predators

The order Aranea was the most abundant order of all predaceous natural enemies within the tree layer with . There were also three other significantly abundant orders. The order Diptera, the order Coleoptera and the order Neuroptera consisted of 597, 246 and 214 individuals of all predaceous individuals respectively. (Figure 10).

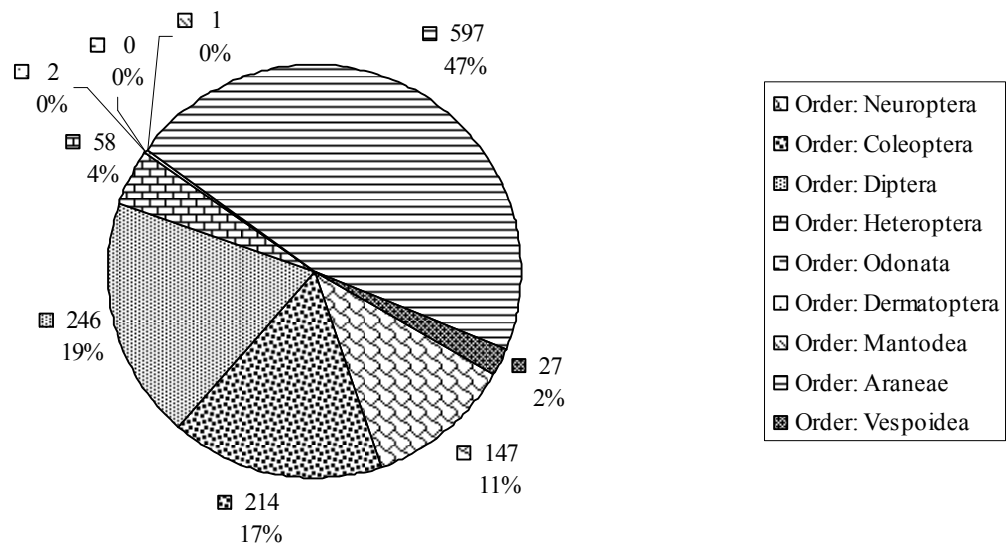


Figure 13. Proportions of the amounts of particular taxa of predaceous natural enemies within tree layer

There were four orders similarly numerous within the cover crop layer. The order Aranea with 535 individuals was also the most abundant of all predaceous taxa, continued closely by the order Diptera, Heteroptera and Coleoptera with 525, 463 and 298 individuals respectively (Figure 11).

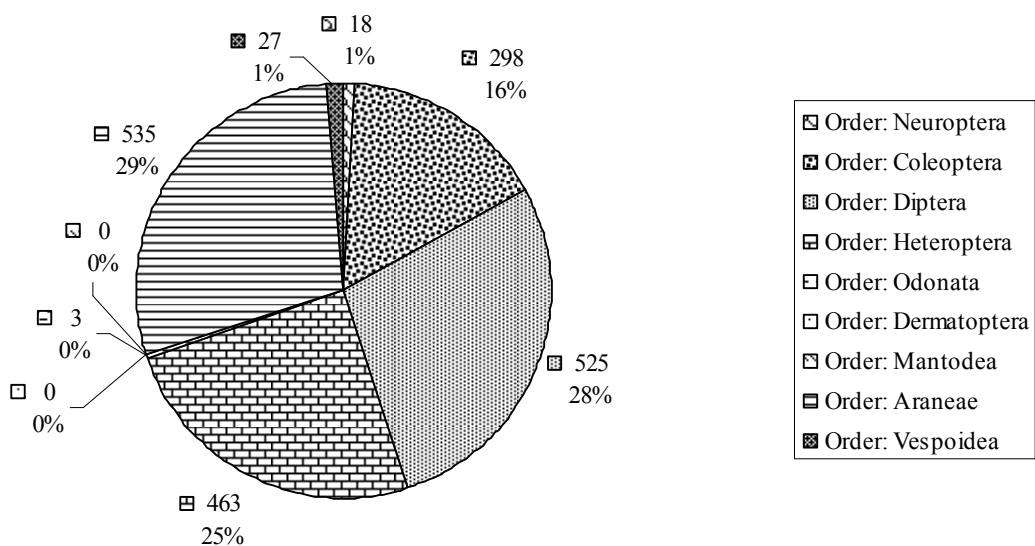


Figure 14. Proportions of the amounts of particular taxa of predaceous natural enemies within the cover crop layer

All considerable numerous orders of natural enemies with predaceous behaviour were likewise captured mostly in cover crop layer (Figure 15).

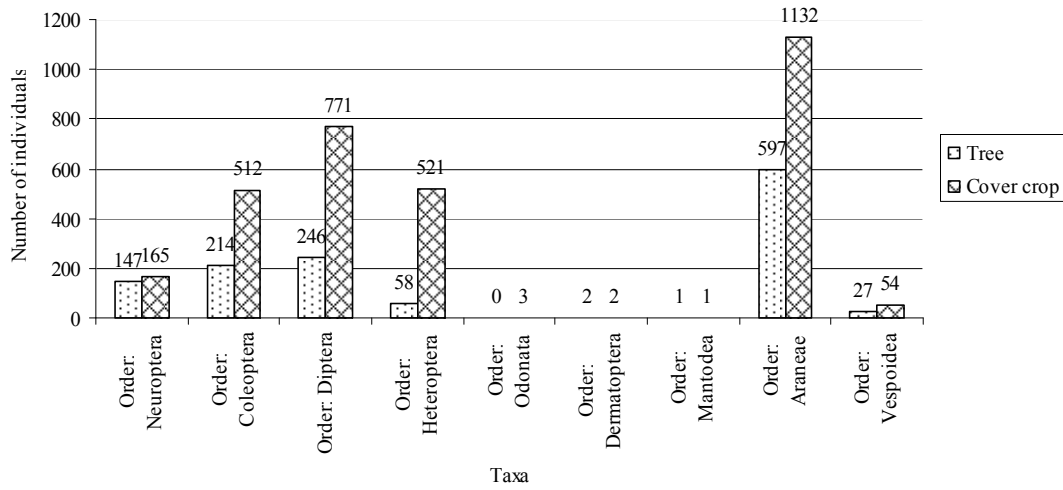


Figure 15. Distribution of the orders of predaceous natural enemies according to vegetation layer

Four most abundant taxa of the order Neuroptera were all considerably more abundant in tree layer (Figure 16).

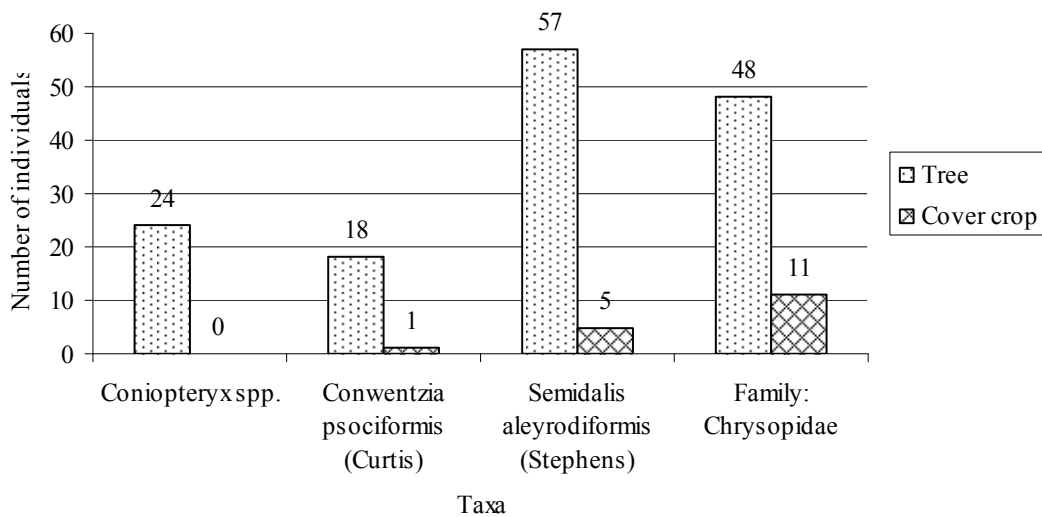


Figure 16. Distribution of the most abundant taxa of the order Neuroptera according to vegetation layer

The families within the order Coleoptera differ in the abundances according to different layers as it is seen in the (Figure 17). The majority of the species of the family Coccidae appears more in tree layer. However, the individuals of *Scymnus*

interruptus (Goeze) are distributed almost equally in both layers. The individuals of the family Staphylinidae were significantly more abundant in cover crop layer.

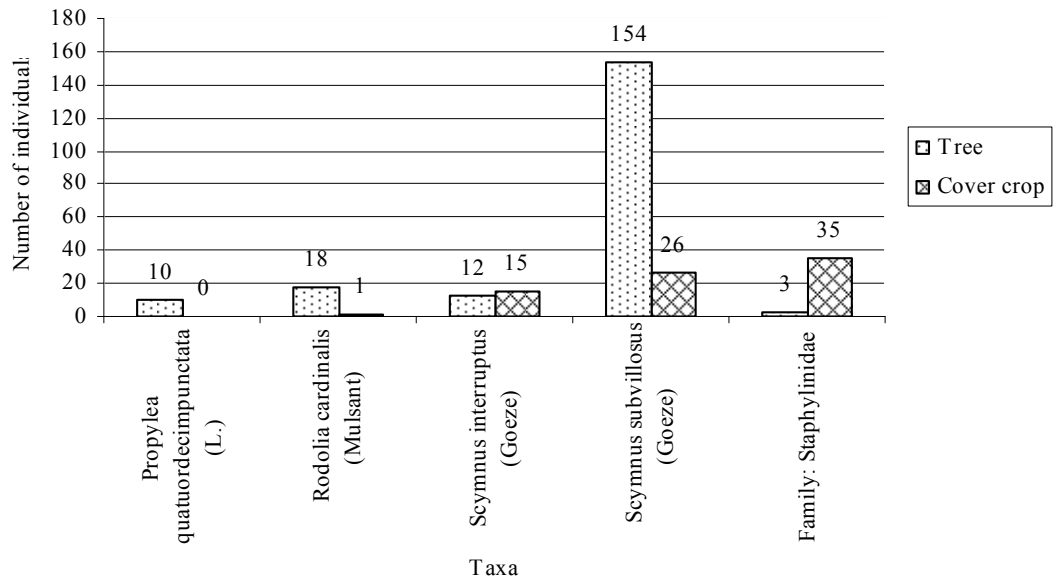


Figure 17. Distribution of the most abundant taxa of the order Coleoptera according to vegetation layer

Within the order diptera there were the taxa Cecidomyiidae and Syrphidae which were both in higher abundance in cover crop layer (Figure 18). The genus *Platypalpus* and the species *Thaumatomyia notata* (Meigen) were not distributed equally, but the difference can be considered as insignificant.

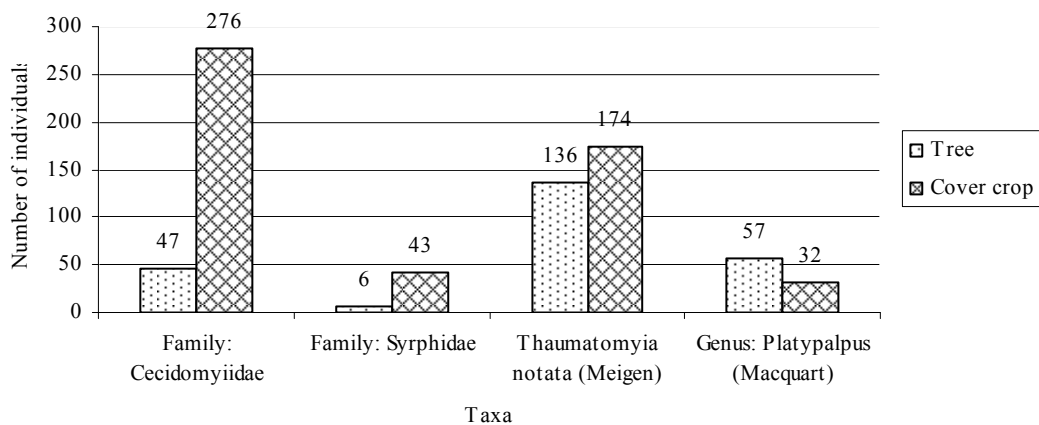


Figure 18. Distribution of the most abundant taxa of the order Diptera according to vegetation layer

Within the order Heteroptera all predaceous taxa except the genus *Pilophorus* (Hahn) and others members of the family Anthocoridae were more considerably more abundant in cover crop layer (Figure 19).

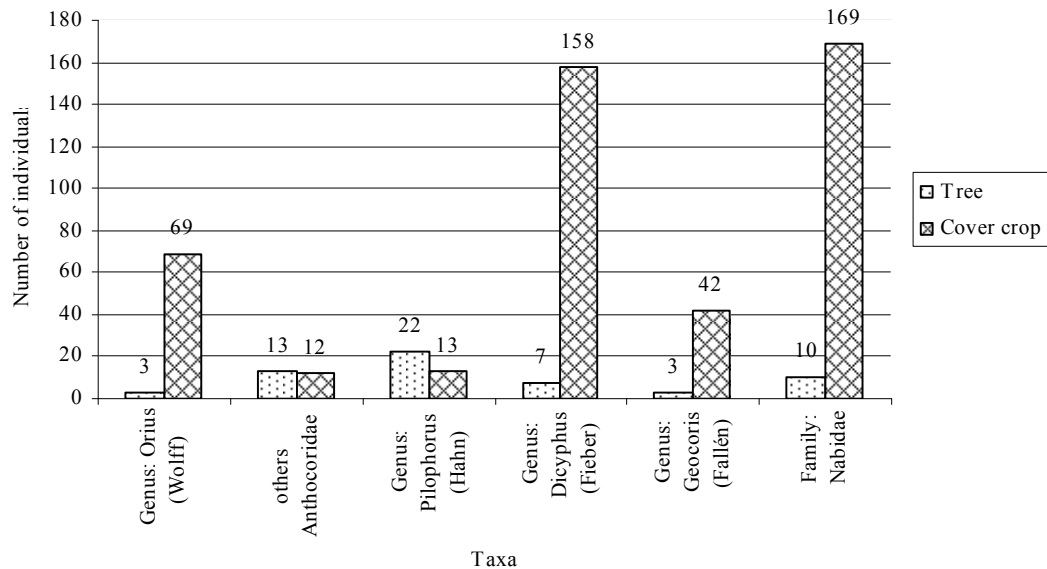


Figure 19. Distribution of the most abundant taxa of the order Heteroptera according to vegetation layer

The amounts of individuals of the order Araneae were very similar in both layers (597 individuals in tree layer and 535 individuals in cover crop layer). Small amounts of the individuals of other taxa of predaceous natural enemies didn't allowed substantial comparison between the abundances in different layers. The individuals of the order Vespoidea were distributed absolutely equally (27 individuals in both tree and cover crop layer).

4.3. Taxa richness and total abundances in different cover crops

The amounts of individuals and the amounts of natural enemy's taxa of all plots with the same cover crop type were fused together for particular layers and whole plots (Table 2).

Table 2. Amounts of natural enemy's individuals and the amounts of natural enemy's taxa of all plots with the same cover crop type were fused together for particular layers and whole plots.

Sample	Cover Crop		Tree		Total	
	No. of taxa	No. of ind.	No. of taxa	No. of ind.	No. of taxa	No. of ind.
Alfalfa	47	1683	53	696	62	2379
Festuca	46	1658	45	531	59	2189
Fest.+Alf.	54	1878	45	551	62	2429
Spontaneous	50	1141	46	578	60	1719

The highest total amount of natural enemies was captured in the plots covered by the Fest.+Alf.cover crop type. There were total number of 2429 individuals captured in these plots. The cover crop type A followed the Fest.+Alf.type closely with 2379 individuals. The third highest abundance of natural enemies, 2189 individuals, had the plots covered by the type F of cover crop. The plots covered by S type of cover crop had the lowest abundance of natural enemies, 1719 individuals. The taxa richness differed of the abundances, but it was very similar between the cover crop types . The plots covered by Fest.+Alf.had total amount of 62 taxa. The plots covered by A type of cover crop had the same number of taxa. The plots covered by S type of cover crop had 60 taxa followed by plots of F type of cover crop with 59 taxa.

Within the tree layer, the greatest number of natural enemies had the plots of A type of cover crop, with 696 individuals. The other types had smaller amounts of individuals similar one to the other. The S type of cover crop was second most abundant with 578 individuals. The Fest.+Alf. type was the third most abundant with 551 individuals of natural enemies and the F type had the least abundance of natural enemies with 531 individuals. The A type of cover crop had the greatest richness of taxa with 53 taxa. The S type of cover crop had considerably lower number of taxa, 46 and both Fest.+Alf. and F types of cover crop had 45 taxa.

Within the cover crop layer, the highest amount of natural enemies had Fest.+Alf. type of cover crop with 1878 individuals. Little less had Fest. and Alf. type of cover crop and the least abundance had S type with 1141 individuals. The highest taxa number had Fest.+Alf. type, with 54 taxa, followed by S type, with 50 taxa. The least number of taxa had plots of F type of cover crop, with 46 taxa. The Alf. type had only one more taxa than the type Fest.

The amounts of individuals and the amounts of taxa of all plots with the same cover crop type were fused together for particular layers and whole plots differing in the distance from the hedge (Table 3).

Table 3. Amounts of natural enemies' individuals and the amounts of natural enemies' taxa of particular plots for particular layers and whole plots

Sample	Cover Crop		Tree		Total	
	No. of taxa	No. of ind.	No. of taxa	No. of ind.	No. of taxa	No. of ind.
Alf. 1	41	829	48	341	58	1170
Alf. 2	42	854	41	355	52	1209
F/F+A	46	821	45	294	58	1115
Fest.+Alf.1	47	905	36	270	55	1175
Fest.+Alf2	41	931	38	281	53	1254
Fest.1	37	871	34	226	48	1097
Fest.2	41	787	43	305	57	1092
Spont.1	40	575	34	274	50	849
Spont.2	45	566	42	304	56	870

The whole plot covered by the Fest.+Alf. type of cover crop based farther from the hedge had the greatest amount of natural enemies with 1254 arthropod individuals. This type of cover crop was followed by the Alf.2 and the Fest.+Alf.1 plots with 1209 and 1175 individuals respectively. The smallest numbers of natural enemies, 870 and 849 individuals, were captured in plots with Spont.2 and Spont.1 respectively. The plot Alf.1 and the plot F/F+A had the highest taxa richness with 58 taxa. The plots Spont.1 and Fest.1 had the least richness of taxa with 50 and 48 taxa respectively.

Within the tree layer, the Alf.2 and the Alf.1 plots had the greatest number of natural enemies with 355 and 341 individuals respectively. The Fest.+Alf.1 and the Fest.1 plots were the ones with the smallest number of individuals with 575 and 566 natural enemies respectively. The Fest.+Alf.1 and the F/F+A plots had the greatest number of taxa within cover crop layer and the plots Spont.1 and Fest.1 had the least number of taxa with 40 and 37 taxa respectively.

Within the cover crop layer, the Fest.+Alf.2 and the Fest.+Alf.1 plots had the highest number of natural enemies with 931 and 905 individuals respectively. The Spont.1 and the Spont.2 plots were the ones with the smallest number of individuals with 575 and 566 natural enemies respectively. The Fest.+Alf.1 and the F/F+A plots

had the greatest number of taxa within cover crop layer and the plots Spont.1 and Fest.1 had the least number of taxa with 40 and 37 taxa respectively.

4.4. Diversity indices

The vast majority of the individuals was identified at least on the taxonomic level of families. Hence, the families were chosen for the comparison of diversity indices. The individuals that were identified only on a higher taxonomical level than family were not used for the calculations of diversity indices calculations. These the orders were Aracnidae, Mantidae, Odonata and the superfamilies Platygastroidea, Proctorupoidea, Cerapronoidea, Vespoidea and Chalcidoidea.

Table 4. Values of the Shannon's, and the Reciprocal Simpson's indices for particular layers of fused plot of same cover crop type

Layer	Tree		Cover Crop		Total	
	H' - Shannon	1/D - Simpson	H' - Shannon	1/D - Simpson	H' - Shannon	1/D - Simpson
Alf.	2.45	7.12	2.02	4.01	2.28	5.41
Fest.	2.33	6.69	2.07	5.18	2.33	6.67
Fest.+Alf.	2.45	7.56	2.36	6.59	2.40	6.89
Spont.	2.38	6.98	2.17	5.10	2.44	7.13

Within the entire fused plots, the plots covered by the Spont. type of cover crop had the highest values of the Shannon's index and the Simpson's reciprocal index (Table 4). The second highest values had the plots with the Fest.+Alf. type of cover crop followed by the type Fest. and Alf. respectively. Within the tree layer, the values of the diversity indices for fused plots were similar to those of entire plots (Table 5). The plots of the Spont. and the Alf. type of cover crop had the highest values of the Shannon's index and the Simpson's reciprocal index. Within the cover crop layer, the plots covered by the Fest.+Alf. type of cover crop had the highest values of the Shannon's index and the Simpson's reciprocal index.

Table 5. Values of the Shannon's, and the Reciprocal Simpson's indices for particular plots and layers

Layer Sample	Tree		Cover Crop		Total	
	H' - Shannon	1/D - Simpson	H' - Shannon	1/D - Simpson	H' - Shannon	1/D - Simpson
Alf.1	2.57	9.63	1.89	3.66	2.25	5.08
Alf.2	2.24	4.92	2.04	4.13	2.27	5.56
Fest.+Alf.1	2.41	8.30	2.18	5.52	2.39	7.00
Fest+Alf2	2.41	6.65	2.12	5.22	2.38	6.71
Fest.1	2.11	5.13	2.08	5.47	2.25	6.39
Fest.2	2.37	7.52	2.11	5.11	2.44	7.16
Spont.1	2.31	7.30	2.15	4.96	2.38	6.63
Spont.2	2.36	7.22	2.18	5.28	2.46	7.49
F/F+A	2.40	6.94	2.06	4.33	2.34	6.14

Among the particular plots of fused layers, the values of diversity indices differed little more (Table 5). The plots with the highest values of the Shannon's index and the Simpson's reciprocal index were the plots with the Spont.2, the Fest.1 and the Fest.+Alf.1 types of cover crop respectively. Both Alf.1 and the Alf.2 types had the lowest values. There were the most considerable differences between the values of diversity indices within the group of plots of tree layer. The plot Alf.1 had the highest value of the all observed indices. According to the value of this the Shannon's index, the plots Fest.+Alf.1 and Fest.+Alf.2 had the second highest values and the plot Fest.1 had the lowest value. According to Simpson's reciprocal index, the plot Fest.+Alf.1 and the plot Fest.2 had the second highest values respectively and the plots Fest.1 and Alf.2 had the lowest values respectively. In the case of particular plots of cover crop layer, the differences were smaller, but still considerable (Table 5). The plot Fest.+Alf.1 had the highest values of all observed indices. The plot Spont.2 had the same value of Shannon's index as the plot Fest.+Alf.1 and the plot Spont.1 had the second highest value of this index. The plot According to Simpson's reciprocal index, the plots Fest.1 and Spont.2 had the second highest value respectively. The plot Alf.1 had the lowest values of all observed indices of diversity.

4.5. Similarity coefficients

For the calculations of similarity coefficients were used all taxa and relevant amounts of individuals. The values were very similar. Nevertheless, the highest value of coefficients for entire plots had Fest. with Fest.+Alf. and the lowest value had

Alf. with Spont. type of cover crop (Table 6). In the case of tree layer Fest. with Spont. had the highest value of coefficient and Fest. with alfalfa had the lowest value (Table 7). In cover crop layer, Fest. with Alf. had the highest value of coefficient but, the differences were very slight (Table 8).

Table 6. Sørensen's and Jaccard's coefficient for entire plots with different cover crop

Plots	Alf.	Fest.+Alf.	Fest.	Spont.
Alf.	x	0.295	0.305	0.295
Fest.+Alf.	0.839	x	0.320	0.303
Fest.	0.876	0.942	x	0.308
Spont	0.836	0.869	0.891	x

Sørensen's coefficient

Jaccard's coefficient

Table 7. Sørensen's and Jaccard's coefficient for cover crop layer on plots with different cover crop

Plots	Alf.	Fest.+Alf.	Fest.	Spont.
Alf.	x	0.290	0.242	0.293
Fest.+Alf.	0.816	x	0.299	0.283
Fest.	0.639	0.854	x	0.308
Spont.	0.828	0.791	0.889	x

Sørensen's coefficient

Jaccard's coefficient

Table 8. Sørensen's and Jaccard's coefficient for tree layer on plots with different cover crop

Plots	Alf.	Fest.+Alf.	Fest.	Spont.
Alf.	x	0.294	0.306	0.287
Fest.+Alf.	0.832	x	0.306	0.293
Fest.	0.882	0.880	x	0.289
Spont.	0.804	0.827	0.813	x

Sørensen's coefficient

Jaccard's coefficient

5. Discussion

The relative abundance of different orders of arthropods were calculated and the results of this study agree with similar recent studies conducted (Aznar *et al.*, 2009; Marco, 2010). Two groups of natural enemies were captured in different layers of the orchard, which permitted to observe habitat behaviour of some numerous species. The family Braconidae comprised almost half of all parasitic natural enemies in this study. They were captured mainly in cover crop layer, this could be the result of their prey specificity. The species of the numerous family Braconidae are parasitoids of many orders within the class Insecta. Nevertheless, some of them are considered hyperparasitoid (García Marí and Ferragut, 2002) hence they could negatively influence parasitism of other parasites by mutualism. The most abundant taxa was the subfamily Opiinae. The members of this family are endoparasitoids of the order Diptera, mostly of the family Tephritidae (Newman). The second most numerous was the subfamily Aphidiinae. The species of this subfamily are specific koinobiont parasitoids of aphids, Aphidoidea (Latreille) (Michelena *et al.*, 2004). The third most numerous group captured were the members of the subfamily Alysiinae which are all parasitoids of flies (Muscomorpha:Diptera) (Trostle *et al.*, 1999). Another numerous group was the subfamily Microgastrinae, whose members host on larvae of Lepidoptera (Walker, 1996) important pests in citrus orchards. The last noticeable taxon was the subfamily Agathidinae with 65 individuals. The members of this family are parasitoids of the order Lepidoptera (Briceño and Sharkey, 2000).

Chalcidoidea comprised only one fourth of all parasitoids in the study but they were the most numerous parasitic taxa within tree layer perhaps due to their prey specificity. The most numerous family was Aphelinidae and the vast majority of them was present in tree layer. They parasitize on eggs of diverse taxa such as scale insects, Coccoidea; greenhouse whiteflies, *Trialeurodes vaporariorum* (Latreille), aphids, Aphidoidea or Psilidae which are pests, present in tree layer. Only few of them were identified as members of the genus *Coccophagus* (Westwood). The members of this genus parasitize mainly on scale insects. (Compere, 1931). The vast majority of individuals was identified only on the level of family but there were some indications that a considerable number of them belonged to the genus *Aphytis*

and *Encarsia* (Foerster) which parasitize on scale insect present in tree layer. Some species of the genus *Aphytis* already proved their great potential for suppression of the California red scale, *Aonidiella aurantii* (Maskell), in citrus orchards in Greece or India (Debach and Argyriou, 1967, Troncho *et al.* , 1992). The members of the genus *Encarsia* (Foerster) also host on the individuals of California red scale (Pina de Montalgrao and Verdú, 2007; Asplanato and Garcia Marí, 2002) or on Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), (Soto *et al.* , 2001) all serious pests of citrus.

The family Encirtidae host on the taxa Coccoidea, Coleoptera, Diptera, Neuroptera, Orthoptera, Araneae, but also on other species of the order Hymenoptera (van Driesche and Bellows, 1996). The most abundant group identified belonged to the genera *Methapycus* (Mercet) The species of this genera are parasitoids of the family Coccidae present in tree layer. They host for example on black scale, *Saissetia oleae* (Olivier), or scale insect, *Coccus hesperidum* L., (Tena *et. al.*, 2007). Mymaridae species have also wide range of their host. They parasitize on taxa Hemiptera, Psocoptera, Coleoptera, Diptera and Orthoptera so their prevalent presence in cover crop layer could be result of host (van Driesche and Bellows, 1996).

The family pteromalidae is very important in BC, particularly in citric orchards. The species of this family parasitise on important pests of citrus. The main host taxa are Mediterranean fruit fly, *Ceratitis capitata* sp.; insect scales *Saissetia* and *Ceroplastes* spp., and *Toxoptera citricidus* sp. (León, 2005). However, they were captured mainly in cover crop layer. It could be explained by migration of their host. *Citrostichus phyllocnistoides* (Narayanan), are very important in control of Citrus leafminer, *Phyllocnistis citrella* Stainton, (Vercher, 2000; Garcia Marí and Ferragut, 2002). Thus its presence in the tree layer was logical.

The family Figitidae parasite especially on larvae of Diptera, but also on the species of the order Neuroptera and Hymenoptera (Ronquist and Nieves-Aldrey, 2001; Buffington, 2008). However, they were found mainly in cover crop layer, so the mutualism doesn't mean to have great effect on pest control by other parasitoids. Within the order Neuroptera, there were identified 3 families. Coniopterygidae. *Coniopteryx* spp., *Conwentzia psociformis* (Curtis) and *Semidalis aleyrodiformis* were not very abundant but their feeding rate is high, so their effect can be

considered very important as they were present basically in tree layer. Recent studies already highlighted the importance of the family Coniopterygidae in the citric orchards, where they feed primarily on the species of taxa Acari (Leach), and Homoptera (Llorens, 1990; Llorens and Garrido, 1992; Ripollés et. al., 1995; Garcia Marí and Ferragut, 2002;) *C. psociformis* feed on important pests of citrus. Its prey are whiteflies, *Aleurothrixus floccosus* (Maskell) (Ripollés and Melià, 1980; Llorens and Garrido, 1992) and the citrus red mite, *Panonychus citri* (McGregor) (García Marí and Ferragut, 2002). According to Monserrat (1984) and Marín and Monserrat (1995), the family Coniopterygidae is the most abundant and species diverse family of the order Neuroptera in the region of Valencia. *Semidalis aleyrodiformis* (Stephens) is believed to be the most common species of this family on the Iberian Peninsula. The members of this species also feed primarily on whiteflies, citrus red mites, but also on red spider mites *Tetranychus urticae* (Koch) (León and Garcia Marí, 2005). There were identified two species within the Green lacewings (Chrysopidae). Common Green Lacewing, *Chrysoperla carnea* (Stephens), comprised the majority and it was present in tree layer almost exclusively. This confirms the opinion that the species *C. carnea* is the most common species of Green lacewings in the citrus orchards in Spain (Bru, 2006). According to Llorens (1990) this species feed especially on citrus red mites but it is also important predator of many aphids.

The beetles (order Coleoptera) is the most diverse order of all (New, 2007). It consist of more than 110 families and many of them are predators. (van Driesche and Bellows, 1996). There were identified 38 individuals as members of the family Staphylinidae in this study. They are believed to be predators of diverse pest species (Jacas and Urbaneja, 2009). The *Cicindela spp.* and soldier beetle, *Rhagonycha fulva* (Scopoli). which also feed on various pests of citrus.

Although, there were identified more families, the most abundant and the most important for the purposes of this study were the members of the family Coccinelidae. There were 258 individuals identified as members of this taxon. It is the most important family of predaceous beetles within the order Coleoptera (DeBach and Rosen, 1991). They primarily feed on whiteflies, *Trialeurodes vaporariorum* (Westwood); the suborder Acari (Leach); scale insects, Coccidae; and especially on aphids, superfamily Aphidoidea (Latreille) (Llorens, 1990, DeBach and

Rosen, 1991, García Marí and Ferragut, 2002). They comprise the family, that was the mostly time introduced of predaceous arthropods in citrus plantations in Spain (Jacas et al., 2006). There were identified 9 taxa of the family Coccinellidae, but only three of them were significantly abundant. The most abundant species was *Scymnus subvillosus* (Goeze) and *Scymnus interruptus* (Goeze). The members of these species primarily feed on aphids. Although, they feed on the same prey they were both found in different layers. It could be explained by the theory „one species one niche” The third most abundant were the members of the species vedalia beetle, *Rodolia cardinalis* (Mulsant). This species was introduced to Spain to control cottony cushion scale, *Icerya purchasi* (Maskell) in 1927 (Alvis et al., 2002). However, the abundance of vedalia beetle could be considered as low in comparison with the study of Orts (2008).

There are two important families within the order. Some of the species of the family Cecidomyiidae feed on aphids, scale insects, whiteflies and mites, Acari (Leach) (Barnes 1929). Nevertheless, there are also some species that are considered as important pest in citrus orchards (García Marí and Ferragut, 2002). The species of the family Syrphidae feed on aphids and they are important especially because they appear during all year (Salveter, 1998). There were also captured individuals of the genus *Platypalpus* spp., but its host is either different from the pest species or not known yet (Markov and Isakulova, 1980; Stark and Wetzell, 1987). Another species of the order diptera, *Thaumatomyia notata* (Meigen) was captured in high abundances. This species is believed to be predator of some aphids (García Marí, 2009).

There were also identified the orders Odonata, Dermaptera and Mantodea. This orders are highly predaceous but their low abundance indicates minor importance within the ecosystem. There were identified individuals of the order Araneae. Although, the vast majority of the species of the order Heteroptera are herbivores and some of them are partially considered as pests, there are also some species that are predators (García Marí and Ferragut, 2002). There were identified 3 taxa which have predaceous behaviour within the whole suborder Heteroptera. *Nabis pseudoferus* sp. was very abundant among the others Heteroptera. This species has been reported as a biological agent of tomato leaf miner, *Tuta absoluta* (Cabello et al., 2009). The genus *Dyciphus* spp. genus *Pilophorus* spp within the family Miridae

are believed to be predators of whiteflies but their abundance was surprisingly very low (Jacas and Urbaneja, 2009)

There were identified members of the family Anthicoridae. Furthermore, the vast majority of them were identified as members of the species *Orius spp.* The species of this family feed on thrips, Thysanoptera (Haliday); other species of the suborder Heteroptera; aphids; species of the family Lepidoptera; and on species of the subclass acari. It is agreed with few other studies, where individuals of this family were also captured in high abundances (Ferragut and González Zamora, 1994; Alvarado et. al., 1997; Riudavets and Castañé, 1998; García Marí and Ferragut, 2002) There were identified members of the genus *Geocoris* (Fallén) within the family Lygaeidae (Schilling) There was great number of individuals identified as members of the superfamily Platigastroidea. The species of this family have broad range of hosts. They host on many taxa of the class Insecta, especially on the species of the orders Lepidoptera, Hemiptera and Orthoptera, but also on some species of the order Araneae. Some of species are considered as a beneficial and they are used in BC. However, there are also some species which host on beneficial cecidomyiid flies, Cecidomyiidae (Gillot) (García Marí and Ferragut, 2002).

The differences in taxa richness were very slight. Krebs (1999) refers to the problematic of distances between plots and plots size. The plots were only about 20 metres one to each other so it is possible that there is strong transition within the agroecosystem. The problem could be also the identification. Many of the members of the order Hymenoptera, which are potential natural enemies can be identified only with specific equipment (Ronquist and Nieves-Aldrey, 2001). However, it could be observed that the diversity indices for natural enemies had higher values for Alf. cover crop and Fest.+Alf. had the highest families richness within the whole plot. Within the tree layer, Alf. had the highest abundance and diversity of natural enemies among the other plots. They seem to be attracted, possibly by flower and they migrate towards the crop. Domínguez Gento *et al.*(2002) observed higher abundance and diversity of natural enemies on the plots of spontaneous species what contrast with this results. Nevertheless, there are few authors that also observed increased abundance within tree layer using cover crop types including alfalfa (García Marí and Ferragut, 2002). The effect of the distance from the hedges was not observed. It

doesn't agree with many studies that claimed, that the presence of hedges favour natural enemies diversity and abundance.

6. Conclusions and recommendations

The vast majority of the agricultural land in developed countries has been for long period managed with chemicals like DDT, which seriously harms the environment and lowers the quality of life. There is a great investment into researches on pest management worldwide. Unfortunately, the vast majority of this economic potential disappears in laboratories on development of new pesticides. Only a small portion of potential flows into the development of sustainable ecologically based agricultural technologies. When speaking about biological control it is obvious that, in the past twenty years, an evident positive progress has been achieved as society tends to support more ecological approaches to agriculture. Unfortunately, there is an urgent need of large scale implementation, which is impossible to be applied without the support of the farmers who can adopt and use these methods. This lack of communication between research and implementation is sometimes called the “death valley”. This problem could find its solution by educating farmers on this field and presenting research from a more approachable perspective. However, it is important to mention that the situation improves year by year. The use of cover crops is an undemanding cultural technique for enhancing natural enemies in order to suppress pest population under the economical level. The ecology of natural enemies and pests arthropods is very complex and it differs rapidly depending on location and conditions. In order to achieve the most accurate results, and before the application of the mentioned methods, it is important to make a profound study of the relationships among the species who share the same ecosystem.

This study was an intention to contribute to scientific discussion on the topic of conservation biological control. In this study there were identified almost 10000 individuals of natural enemies which were captured in almost twenty repetitions of samplings. This high number of individuals and relatively high number of repetitions have been promising that the data will be valuable for assessment. As mentioned before, the results of this study show that alfalfa, pure or mixed with another plant species, could be recommended as a good candidate for further and more specific studies on conservation biological control.

It could also be interesting to observe how the alteration of the dimensions of plots and the distances of the hedges would affect the results.

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7. Supplements

Annex 1. Taxonomy, ecological function, and distribution of identified arthropods

Taxonomy	Function	Tree	Cover crop	Total
SUBPHYLUM: HEXAPODA		8,716	35,175	43,891
ORDER: NEUROPTERA	Predator	147	18	165
Family: Coniopterygidae	Predator	99	6	105
<i>Coniopteryx</i> spp.	Predator	24	0	24
<i>Conwentzia psociformis</i> Curtis	Predator	18	1	19
<i>Semidalis aleyrodiformis</i> Stephens	Predator	57	5	62
Family: Chrysopidae	Predator	48	11	59
<i>Chrysopa septempunctata</i> Westmael	Predator	3	0	3
<i>Chrysoperla carnea</i> Stephens	Predator	45	10	55
Family: Hemerobiidae	Predator	0	1	1
ORDER: COLEOPTERA		296	304	600
Family: Coccinellidae	Predator	210	48	258
<i>Propylea quatuordecimpunctata</i> L.	Predator	10	0	10
<i>Rodolia cardinalis</i> Mulsant	Predator	18	1	19
<i>Scymnus interruptus</i> Goeze	Predator	12	15	27
<i>Scymnus subvillosus</i> Goeze	Predator	154	26	180
<i>Stethorus punctillum</i> Weise	Predator	3	1	4
<i>Rhyzobius lophantae</i> Blaisdell	Predator	2	0	2
<i>Coccinella septempunctata</i> L.	Predator	2	0	2
<i>Rhizobius littura</i> Fabr.	Predator	0	4	4
others	Predator	9	1	10
Family: Curculionidae	Herbivore	7	26	33
Family: Staphylinidae	Predator	3	35	38
Family: Mordellidae	Unspecified	0	4	4
others Coleoptera		76	191	267
<i>Olibrus affinis</i> Sturm	Unspecified	12	41	53
<i>Corylophidae</i> spp.	Herbivore	12	40	52
<i>Arthrolips obscurus</i> Sahlberg	Herbivore	10	51	61
<i>Rhagonycha fulva</i> Scopoli	Predator	0	1	1
<i>Cicindela</i> spp.	Predator	1	0	1
others	Unspecified	41	58	99

Taxonomy	Function	Tree	Cover crop	Total
ORDER: DIPTERA		263	531	794
Family: Cecidomyiidae	Predator	47	276	323
Family: Syrphidae	Predator	6	43	49
<i>Sphaerophoria</i> spp.	Predator	1	16	17
<i>Syrphus</i> spp.	Predator	0	1	1
others	Predator	5	26	31
Family: Psychodidae	Unspecified	11	4	15
Family: Chironomidae	Unspecified	2	0	2
<i>Thaumatomyia notata</i> Meigen	Predator	136	174	310
Genus: Platypalpus	Predator	57	32	89
others Diptera	Unspecified	4	2	6
SUBORDER: HETEROPTERA		216	4844	5,060
Family: Anthocoridae	Predator	16	81	97
<i>Orius</i> spp.	Predator	3	69	72
others	Predator	13	12	25
Family: Miridae		76	842	918
<i>Adelphocoris</i> <i>lineolatus</i> Goeze	Herbivore	24	513	537
<i>Pilophorus</i> sp.	Predator	22	13	35
<i>Dyciphus</i> sp.	Predator	7	158	165
<i>Lygus pratensis</i> L.	Herbivore	11	29	40
others	Unspecified	12	129	141
Family: Lygaeidae	Herbivore	86	3480	3,566
<i>Beosus maritimus</i> Scopoli	Herbivore	0	1	1
<i>Nysius</i> sp.	Herbivore	81	3421	3,502
<i>Geocoris</i> sp.	Herbivore	3	42	45
<i>Lygaeus equestris</i> L.	Herbivore	0	1	1
others	Herbivore	2	15	17
Family: Nabidae	Predator	10	169	179
<i>Nabis pseudoferus</i> Remane	Predator	10	168	178
others	Predator	0	1	1
Family: Alydidae	Herbivore	0	3	3
Family: Pentatomidae	Herbivore	7	12	19
others Heteroptera	Unspecified	21	257	278
ORDER: HYMENOPTERA		2,439	6,536	8,975
Family: Formicidae	Predator	978	753	1,731
Superfamily: Ichneumonoidea	Parasitoid	238	3,160	3,398
Family: Braconidae	Parasitoid	199	2873	3,072
Subfamily: Rogadinae	Parasitoid	2	0	2
Subfamily: Homolobinae	Parasitoid	4	23	27
Subfamily: Horminae	Parasitoid	0	2	2

Taxonomy	Function	Tree	Cover crop	Total
Subfamily: Euphorinae	Parasitoid	7	10	17
Subfamily: Braconinae	Parasitoid	0	16	16
Subfamily: Agathidinae	Parasitoid	4	61	65
Subfamily: Microgastrinae	Parasitoid	12	124	136
Subfamily: Aphidiinae	Parasitoid	33	727	760
Subfamily: Opiinae	Parasitoid	90	1544	1,634
Subfamily: Alysiinae	Parasitoid	47	366	413
Tribe: Alysiini	Parasitoid	42	250	292
Tribe: Dacnusiini	Parasitoid	5	116	121
Family: Icheumonidae	Parasitoid	39	287	326
Family: Figitidae	Parasitoid/Hyperparasitoid	28	283	311
Superfamily: Ceraphronoidea	Parasitoid/Hyperparasitoid	46	120	166
Family: Ceraphronidae	Parasitoid/Hyperparasitoid	38	47	85
Family: Megaspilidae	Parasitoid/Hyperparasitoid	0	34	34
others	Parasitoid	8	39	47
Superfamily: Platygastroidea	Parasitoid	213	829	1,042
<i>Inostemma sp.</i>	Parasitoid	2	42	44
<i>Synopeas sp.</i>	Parasitoid	28	48	76
others	Parasitoid	183	739	922
Superfamily: Chalcidoidea	Parasitoid	810	1,045	1,855
Family: Aphelinidae	Parasitoid	367	48	415
Coccophagus sp.	Parasitoid	17	3	20
others	Parasitoid	350	45	395
Family: Eulophidae	Parasitoid	152	282	434
<i>Citrostichus phyllocnistoides</i>	Parasitoid	85	4	89
Narayanan				
others	Parasitoid	67	278	345
Family: Encirtidae	Parasitoid	112	50	162
<i>Metaphycus sp.</i>	Parasitoid	64	2	66
<i>Microteris nietneri</i>	Parasitoid	10	1	11
others	Parasitoid	38	47	85
Family: Pteromalidae	Parasitoid	126	302	428
Family: Mymaridae	Parasitoid	25	242	267
Family: Trichogrammatidae	Parasitoid	2	11	13
Family: Chalcididae	Parasitoid	2	2	4
others Chalcidoidea	Parasitoid	24	108	132
others Hymenoptera		126	346	472
Order: Vespoidea	Predator	27	27	54
Superfamily: Proctorupoidea	Parasitoid	15	82	97

Taxonomy	Function	Tree	Cover crop	Total
Family: Chrysidae	Parasitoid	0	2	2
Family: Argidae	Herbivore	2	6	8
others	Unspecified	82	229	311
ORDER: LEPIDOPTERA	Herbivore	219	130	349
<i>Phyllocnistis citrella</i>	Herbivore	100	20	120
Stainton				
others Lepidoptera	Herbivore	119	110	229
SUBORDER: HOMOPTERA	Herbivore	2,673	20,052	22,725
<i>Empoasca</i> sp.	Herbivore	143	1,542	1,685
Family: Delphacidae	Herbivore	148	4704	4,852
Family: Aleyrodidae	Herbivore	362	38	400
Family: Aphididae	Herbivore	1,537	6,927	8,464
Family: Cicadellidae	Herbivore	483	6,841	7,324
others HEXAPODA		2463	2760	
Order: Thysanoptera	Herbivore	87	705	792
Order: Psocoptera	Detrivore	657	80	737
Order: Odonata	Predator	0	3	3
Order: Dermaptera	Predator	2	0	2
Subclass: Collembola	Herbivore	3	1655	1,658
Family: Coccidae	Herbivore	1677	92	1,769
Order: Ephemeroptera	Unspecified	7	1	8
Family: Mantidae	Predator	1	0	1
Order: Orthoptera	Herbivore	29	224	253
SUBPHYLUM: CRUSTACEA		0	6	6
Order: Isopoda	Unspecified	0	6	6
CLASS: ARACHNIDA		926	2,801	3727
Order: Araneae	Predator	597	535	1132
Subclass: Acari	Herbivore	329	2266	2595
TOTAL		9,642	37,982	47624