

Contribution of marginal non-crop vegetation and semi-natural habitats to the regulation of insect pests populations by their natural enemies

Luan Alberto Odorizzi dos Santos

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**UNIVERSIDADE ESTADUAL PAULISTA “JULIO DE MESQUITA FILHO”
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL**

**CONTRIBUTION OF MARGINAL NON-CROP
VEGETATION AND SEMI-NATURAL HABITATS TO THE
REGULATION OF INSECT PEST POPULATIONS BY THEIR
NATURAL ENEMIES**

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Biólogo

Jaboticabal - São Paulo - Brasil

2017

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Orientador: Prof. Dr. Odair Aparecido Fernandes

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias- UNESP Câmpus de Jaboticabal como parte das exigências para a obtenção do título de Doutor em Agronomia (Entomologia Agrícola)

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DADOS CURRICULARES DO AUTOR

LUAN ALBERTO ODORIZZI DOS SANTOS- Nasceu em 29 de agosto de 1988, na cidade de Cândido Mota, SP. Formou-se em Licenciatura e Bacharel em Ciências Biológicas (2011) na Universidade Estadual do Norte do Paraná, Câmpus Luiz Meneghel, Bandeirantes, PR. Durante a graduação trabalhou no Laboratório de Nematologia e Entomologia, sob supervisão da Prof^a. MSc Nina Maria Silva Risso e orientação da Prof^a. Dr^a. Laila Herta Mihsfeld, atuando em projetos com controle biológico de *Diatraea saccharalis*, dispersão de *Cotesia flavipes* e práticas laboratoriais em Nematologia Agrícola. Em agosto de 2011, ingressou no mestrado do programa de Entomologia Agrícola da Faculdade de Ciências Agrárias e Veterinárias, Câmpus de Jaboticabal, sendo bolsista CAPES, no Laboratório de Ecologia Aplicada, sob orientação do Prof. Dr. Odair Aparecido Fernandes. Em agosto de 2013 ingressou no doutorado pelo mesmo programa e orientador. Por meio de parcerias internacionais e financiamento da CAPES, desenvolveu parte dos seus estudos doutorais na França no período de Abril/2015 à Março/2016 sob supervisão do Prof. Dr. Armin Bischoff pela Université d'Avignon et Pays de Vaucluse. Ainda nesse período desenvolveu trabalhos no INRA (Avignon) sob supervisão dos professores Dr. Pierre Franck e Dr^a. Claire Lavigne.

“Tu te tornas eternamente responsável por aquilo que cativas.”

(Antoine de Saint-Exupéry)

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RESUMO GERAL

A expansão das áreas agrícolas levou à perda de biodiversidade devido à redução dos habitats naturais e semi-naturais nas paisagens agrícolas. Com o aumento da produção agrícola no mundo, são cada vez mais discutidas técnicas ambientais que permitem uma gestão sustentável dos habitats do entorno. Os efeitos desses habitats sobre a população de insetos-pragas e seus inimigos naturais ainda são mal compreendidos. O objetivo desta tese foi compreender os efeitos dos ambientes naturais e semi-naturais na população de insetos-pragas e inimigos naturais nas regiões tropicais (Brasil) e temperadas (França). No Brasil (Capítulo II) foi avaliado o efeito da distância de fragmentos sobre a população de formigas predadoras e omnívoras em cana-de-açúcar. Os resultados mostraram que a riqueza de espécies diminui com a distância dos fragmentos florestais e que a dominância das espécies *Dorymyrmex bruneus* e *Pheidole oxyops* aumenta. As espécies de formigas que colonizam áreas de cana-de-açúcar também foram encontradas em fragmentos de florestas, sugerindo que estes últimos são abrigos para espécies de formigas predatórias durante períodos de perturbação como colheita de cana-de-açúcar ou preparo do solo. Isto foi confirmado por diferenças mais fortes nas comunidades de formigas após a colheita da cana (estação seca) do que quatro meses depois (estação chuvosa) quando a ausência de perturbação permitiu recolonização por formigas. Houve também uma diferença na riqueza de espécies de formigas entre diferentes tipos de fragmentos (vales de rios e planícies). Na França, foi avaliado o efeito de faixas de flores silvestres, vegetação espontânea e gramíneas na comunidade de inimigos naturais e a regulação do pulgão cinza *Dysaphis plantaginaea* (Capítulo III). No que diz respeito aos principais inimigos naturais, os nossos resultados mostraram uma maior densidade de sirfídeos nas faixas de flores em comparação com outros tipos de faixas, mas nenhuma diferença para joaninha (Coccinellidae). Não foram observadas diferenças nas

densidades de inimigos naturais dentro dos pomares. O número de afídeos foi maior perto das margens, sugerindo que a colonização das faixas nas margens pode neutralizar os efeitos reguladores positivos dos inimigos naturais. Os efeitos positivos da vegetação nas faixas da margem na regulação de pragas da maçã requerem um movimento de inimigos naturais no pomar. Nós testamos os movimentos de predadores generalistas marcando as faixas das margens com proteína de ovo e verificamos se eles se alimentavam de insetos pragas usando a análise de marcadores moleculares de genes de mariposas (*Cydia pomonella*) nos predadores (capítulo IV). Os resultados mostraram que poucos indivíduos estavam se movimentando das margens do campo para o pomar. No entanto, 25% dos predadores capturados alimentados com *C. pomonella* ainda indicam um alto nível de regulação natural. Em conclusão, o movimento limitado de predadores das faixas de plantas nas margens em pomares pode explicar a ausência de diferenças entre os tratamentos na regulação de pragas do pomar (pulgões). Habitats naturais e semi-naturais podem contribuir para o controle de pragas nas margens das culturas, mas em pomares de maçã este efeito diminuiu fortemente com a distância.

GENERAL ABSTRACT

The expansion of agricultural areas has led to the loss of biodiversity due to the reduction of natural and semi-natural habitats in agricultural landscapes. With the increase of agricultural production in the world, environmentally sound techniques are increasingly discussed that allow a sustainable management of surrounding habitats. The effects of these habitats on the population of insect pests and their natural enemies are still poorly understood. The objective of this thesis was to understand the effects of natural and semi-natural environments on the population of insect pests and natural enemies in tropical (Brazil) and temperate (France) regions. In Brazil (Chapter II) the effect of the distance of fragments on the population of predatory and omnivorous ants in sugarcane was evaluated. The results showed that the species richness decrease with distance from forest fragments and that the dominance of the species *Dorymyrmex bruneus* and *Pheidole oxyops* increase. Ant species colonizing sugarcane fields were also found in forest fragments suggesting that the latter habitats are shelters for predatory ant species during periods of disturbance such as sugarcane harvest or soil tillage. This was confirmed by stronger differences in ant communities after sugarcane harvest (dry season) than four months later (rainy season) when absence of disturbance allowed re-colonization by ants. There was also a difference in the richness of ant species between different fragment types (river valleys and plateaus). In France, the effect of wildflower strips, spontaneous vegetation and grass strips on the community of natural enemies and the regulation of the rosy apple aphid *Dysaphis plantaginaea* (Chapter III) were evaluated. Concerning major natural enemies, our results showed a higher density of hoverflies compared with other strip types but no difference for ladybirds (coccinellidae). No differences in natural enemy densities were observed inside orchards. Aphid number was higher close to the margins suggesting that colonization from margin strips may counteract positive regulatory effects of natural enemies. Positive effects of strip margin vegetation on

regulation of apple pests require a movement of natural enemies into the orchard. We tested the movements of generalist predators by labelling margin strips with egg protein and we checked whether they fed on pest insects using genetic marker analysis of codling (*Cydia pomonella*) moth genes inside predators (chapter IV). The results showed that few individuals were moving from the field margins into the orchard. However, 25% of the captured predators fed on *C. pomonella* still indicating a high level of natural regulation. In conclusion, the limited movement of predators from margin strips into orchards may explain the absence of differences between strip treatments in orchard pest regulation (aphids). Natural and semi-natural habitats can contribute to pest control at the margins of crops, but in apple orchards this effect strongly decreased with distance.

RÉSUMÉ GENERAL

L'expansion des zones agricoles a conduit à la perte de biodiversité due à la réduction des habitats naturels et semi-naturels dans les paysages agricoles. Avec l'augmentation de la production agricole dans le monde, des techniques écologiquement rationnelles sont de plus en plus discutées qui permettent une gestion durable des habitats environnants. Les effets de ces habitats sur la population des insectes nuisibles et de leurs ennemis naturels sont encore mal connus. L'objectif de cette thèse était de comprendre les effets des environnements naturels et semi-naturels sur la population des insectes nuisibles et des ennemis naturels dans les régions tropicales (Brésil) et tempérées (France). Au Brésil (chapitre II), on a évalué l'effet de la distance des fragments sur la population de fourmis prédatrices et omnivores dans la canne à sucre. Les résultats montrent que la richesse en espèces diminue avec la distance des fragments forestiers et que la prédominance des espèces *Dorymyrmex brunneus* et *Pheidole oxyops* augmente. Des espèces de fourmis colonisant les champs de canne à sucre ont également été trouvées dans des fragments forestiers, ce qui suggère que ces derniers habitats sont des refuges pour les espèces de fourmis prédatrices pendant les périodes de perturbation comme la récolte de la canne à sucre ou le travail du sol. Cela a été confirmé par des différences plus fortes dans les communautés de fourmis après la récolte de la canne à sucre (saison sèche) que quatre mois plus tard / (saison des pluies) quand l'absence de perturbation a permis la re-colonisation par les fourmis. Il y avait aussi une différence dans la richesse des espèces de fourmis entre les différents types de fragments (vallées fluviales et plaines). En France, on a évalué l'effet des bandes de fleurs sauvages, de la végétation spontanée et des bandes d'herbe sur la communauté des ennemis naturels et la régulation du puceron de la pomme rosâtre *Dysaphis plantaginaea* (chapitre III). En ce qui concerne les principaux ennemis naturels, nos résultats ont montré une densité plus élevée de hoverflies par rapport à d'autres types de bande, mais aucune différence pour les coccinelles (coccinellidae).

Cependant, aucune différence de densité naturelle de l'ennemi n'a été observée à l'intérieur des vergers. Le nombre de pucerons était plus élevé près des marges, ce qui suggère que la colonisation à partir des bandes marginales peut contrecarrer les effets régulateurs positifs des ennemis naturels. Les effets positifs de la végétation de la marge de bande sur la régulation des ravageurs de la pomme nécessitent un mouvement d'ennemis naturels dans le verger. Nous avons testé les mouvements des prédateurs généralistes en étiquetant les bandes de marge avec des protéines d'œufs et nous avons vérifié si elles se nourrissaient d'insectes nuisibles en utilisant l'analyse de marqueurs génétiques des gènes de papillon (*Cydia pomonella*) à l'intérieur des prédateurs (chapitre IV). Les résultats ont montré que peu de personnes se déplaçaient des marges de champ dans le verger. Cependant, 25% des prédateurs capturés se nourrissaient de *C. pomonella* indiquant encore un haut niveau de régulation naturelle. En conclusion, le mouvement limité des prédateurs à partir des bandes de marges dans les vergers peut expliquer l'absence de différences entre les traitements de bandes dans la régulation des ravageurs du verger (pucerons). Les habitats naturels et semi-naturels peuvent contribuer à la lutte contre les ravageurs en marge des cultures, mais dans les vergers de pommiers cet effet a fortement diminué avec la distance.

CHAPTER I- GENERAL INTRODUCTION

The planet earth is experiencing mass extinction due to species loss at local and global level (Thomas et al., 2004). The expansion of modern agriculture and intensive use of chemicals have been identified as one of the greatest threats to biodiversity (Tilman et al., 2001), and therefore to extinction of even unknown species. These losses have promoted initiatives to increase biodiversity in agricultural environments (Hole et al., 2005).

Conservation biological control (CBC) by habitat management is discussed as a strategy to restore biodiversity of agro-ecosystems providing a wide variety of ecosystem services such as pest reduction without harming the environment. A better understanding of the ecology of natural enemies along with biotic and abiotic factors is required for the success of biological control programs, since these interactions have a strong impact on the population dynamics of prey and hosts (Pearce et al., 2006). Conservation biological control (CBC) is based on the management of habitats with the purpose of increasing the abundance of natural enemies in agro-ecosystems and improving the efficiency of insect pest regulation (Gurr and Wratten, 2000; Gurr et al., 2004). Habitat management for CBC provides food and shelter to natural enemies which are of fundamental importance for their maintenance during seasons when crops and pests are absent. Beetle banks provide for example such a shelter function. They are usually grass mixtures sown as marginal or within-crop strips (Thomas et al., 1991, Collins et al. 2002). These strips provide suitable overwintering habitats for ground and rove beetles. Other examples of CBC are well documented (Landis et al., 2000; Gurr et al. 2004). In general, predators and parasitoids depend on few prey or host species to complete their life cycles (Coll and Guershon, 2002; Wäckers et al., 2005). However, plant resources of semi-natural habitats are of fundamental importance to natural enemies because they may change nutritional requirements during their life cycle. Hoverflies, ladybirds, lacewings, for instance,

use pollen as adults though larvae are predators. Thus, entomophilous plant species providing pollen and nectar increase food resource availability and, consequently, the abundance of natural enemies in agroecosystems. Therefore, wildflower strips are currently studied to increase predation and / or parasitism rates and to reduce pest populations (Haaland et al. 2011). Wildflower strips are a mixture of predominantly entomophilous plants sown to the margins edges of agricultural fields in order to attract and to increase the abundance and performance of arthropods groups related to different ecosystem services, such as pollination and biological control (e.g.; bees, butterflies, and natural enemies). In their review on wildflower strips, Fiedler et al. (2008) reported that more than 35 botanical families are used in such plantations to attract biological control agents, and that Apiaceae, Asteraceae, Fabaceae, and Lamiaceae were the most common families.

In order to identify the most appropriate plant species, floral nectar amount and quality, floral morphology, plant phenology and food preference of the major natural enemies need to be considered (Campbell et al., 2012; Wäckers; van Rijn, 2012). Parasitoids, for example, require sugar resources such as floral nectar to meet their nutritional needs (Wäckers, 2004). Moreover, these resources not only increase the longevity of adult parasitoids (Winkler et al., 2009), but also increase their efficiency to reduce pest populations (Winkler et al., 2006). Thus the choice of plant species largely determines the biological control success. Additionally, plant species of wildflower strips should not compete with crops for resources and should not favor insect pests (Bukovinsky et al., 2004).

Studies on plant species composition and the importance of entomophilous plants in pest regulation are less frequent than those examining the influence of natural and semi-natural habitats. Therefore, there is a lack of information on the former issue (Bischoff et al., 2016).

In addition to herbaceous vegetation, forest fragments such as Brazilian permanent conservation areas, adjacent to crops, may provide resources for natural enemies facilitating their movement into crop fields, as suggested by Thomas et al. (1991). However, several arthropod groups and/or species are confined to either crop or natural habitats (Duelli et al., 1990, Baldissera et al., 2004). Arthropods restricted to natural habitats of agricultural landscapes are denominated "stenotypic species" (Duelli and Obrist, 2003). These species (eg, carabid beetles restricted to woody habitats) are rarely found in crop fields. On the other hand, "cultural species" strongly prefer agricultural crops and generally do not depend on semi-natural habitats (Duelli and Obrist, 2003).

In Brazil, the Atlantic Forest, occupied more than one million square kilometers, but today it was reduced to 12% of the original coverage (Ribeiro et al., 2009) and a total protected area of only 1.62% (Laurance, 2009). It is known that habitat structure is important for the tropical ant community (Lassau and Hochuli, 2004), and there is a strong association between ant species richness / composition and a complex habitat structure in regenerating forests (Dunn, 2004). Thus, ants are usually used as bioindicators of ecological changes and ecosystem dynamics (Underwood and Fisher, 2006) because they are highly abundant, diverse and respond to ecosystem disturbances. Ants are also important predators, competitors, and mutualists in most terrestrial habitats (Hölldobler and Wilson, 1990). More recently, ants have been discussed as important agents of biological control in crop fields regulating crop pest insects (Offenberg 2015). However, our knowledge on the role of natural habitats such as forest fragments as refuge for predatory ants attacking pest insects is very poor and at present we do not know whether forest fragments contribute to natural regulation.

Several natural enemies of pest insects switch between (semi-)natural habitats and crop fields (Thies and Tschardtke, 1999; Bianchi et al., 2006). Often, such arthropods feed within crop fields, but return to semi-natural habitats more protected against disturbance or

their own natural enemies (Landis et al., 2000, Rand et al., 2006). In order to evaluate the importance of such semi-natural habitats in crop pest insect regulation a better knowledge on frequency and distance of movements is required.

The present thesis aimed at evaluating the role of different non-crop habitat types in regulating pest insects of different crops. I worked in apple orchards (France) and sugar cane (Brazil) because they are two important agricultural crops in these countries. In France, the effect of wildflower strips on pest regulation (aphids) and on conservation of natural enemies (spiders, ground beetles, and parasitic wasps) was studied. In Brazil, as ants play an important role in sugarcane pest regulation, the contribution of semi-natural areas and forest fragments to ants communities was analyzed.

In the first part I analyzed the effects of different herbaceous margin strips on rosy apple aphids and their natural enemies in European apple orchards. In the second part I examined the influence of forest fragments on predatory ant species regulating lepidopteran pest species in sugarcane fields. In the third part I tried to understand the movement of predators between semi-natural habitats and crop fields (orchards) as well as their effects on population regulation.

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CHAPTER II - THE EFFECT OF FOREST FRAGMENTS ON ABUNDANCE, DIVERSITY AND SPECIES COMPOSITION OF PREDATORY ANTS IN SUGARCANE FIELDS

RESUMO

A perda e a fragmentação do habitat provocaram gradualmente a perda de diversidade e, conseqüentemente, o declínio dos serviços ecológicos. O objetivo deste trabalho foi avaliar o efeito dos habitats naturais e semi-naturais (fragmentos de vale do rio e fragmentos florestais) sobre a comunidade de formigas predadoras e omnívoras em canaviais. Vinte áreas (10 de cada tipo de fragmento) foram selecionadas e avaliadas diretamente após a colheita (outubro de 2014, estação seca) e no meio da estação de crescimento (janeiro de 2015, estação das chuvas). Em cada área, as formigas foram amostradas em cinco parcelas lineares (10 m dentro do fragmento, 0 m [trajeto de campo entre o campo e o fragmento], 5 m, 50 m e 100 m dentro da cultura). Cada parcela compreendia 10 iscas de sardinha numa linha paralela à borda do campo. As iscas foram expostas durante 30 minutos e as formigas foram levadas ao laboratório para identificação. A riqueza de espécies e a frequência de espécies de formigas diminuíram com o aumento da distância dos fragmentos florestais. Dentro dos campos, a riqueza e a frequência de espécies foram maiores durante o período de crescimento vegetativo (estação de chuva) do que após a colheita (estação seca). A comunidade de formigas foi mais semelhante em cana-de-açúcar e fragmentos florestais pós-perturbação pela colheita da cana sugerindo uma recolonização a partir dos fragmentos para o canavial. As espécies dominantes *Dorymyrmex brunneus* e *Pheidole oxyops* foram frequentemente observadas em fragmentos e campos de cultivo, indicando que os fragmentos apresentam potencial para contribuir com a re-colonização por formigas predatórias e conseqüentemente com o controle biológico de pragas da cana-de-açúcar.

ABSTRACT

Habitat loss and fragmentation has gradually caused the loss of diversity and consequently the decline of ecological services. This study aimed to evaluate the effect of natural and semi-natural habitats (river valley fragments and forest fragments) on the community of predatory and omnivorous ants in sugarcane fields. Twenty areas (10 from each fragment type) were selected and evaluated directly after harvest (October 2014, dry season) and in the middle of the growing season (January 2015, rainy season). In each area, ants were sampled in five linear plots (10 m inside the fragment, 0 m [field path between field and fragment], 5 m, 50 m and 100 m inside the culture). Each plot comprised 10 sardine baits in a row parallel to the field edge. The baits were exposed for 30 minutes and ants were taken to the laboratory for identification. Species richness and frequency of ant species decreased with increasing distance from the forest fragments. Inside fields, species richness and abundance were higher during the period of vegetative growth (rain season) than after harvest (dry season). Ant communities of sugarcane fields and forest fragments were more similar later in the season than directly after disturbance by sugarcane harvest suggesting a recolonization from fragments into fields. The dominant species *Dorymyrmex brunneus* and *Pheidole oxyops* were frequently observed in fragments and crop fields confirming the potential contribution of fragments to the recolonization processes and therefore to biological control of sugarcane pest insects.

RÉSUMÉ

La perte et la fragmentation de l'habitat ont peu à peu causé la perte de diversité et, par conséquent, le déclin des services écologiques. Ce travail a pour objectif d'évaluer l'effet des habitats naturels et semi-naturels (fragments de vallées et fragments forestiers) sur la communauté des fourmis prédatrices et omnivores dans les champs de canne à sucre. Vingt secteurs (10 de chaque type de fragment) ont été sélectionnés et évalués directement après la récolte (octobre 2014, saison sèche) et au milieu de la saison de croissance (janvier 2015, saison des pluies). Dans chaque zone, les fourmis ont été échantillonnées dans cinq parcelles linéaires (10 m à l'intérieur du fragment, 0 m [parcours entre champ et fragment], 5 m, 50 m et 100 m à l'intérieur de la culture). Chaque parcelle comprenait 10 appâts de sardine dans une rangée parallèle au bord du champ. Les appâts ont été exposés pendant 30 minutes et les fourmis ont été emmenées au laboratoire pour identification. La richesse en espèces et la fréquence des espèces de fourmis ont diminué avec l'augmentation de la distance à partir des fragments de forêt. À l'intérieur des champs, la richesse et l'abondance des espèces étaient plus élevées au milieu de la saison de croissance (saison des pluies) qu'après la récolte (saison sèche). Communautés de fourmis des champs de canne à sucre et des fragments forestiers étaient plus similaires plus tard dans la saison que directement après la perturbation par la récolte de la canne à sucre suggérant la recolonisation de fragments dans les champs. Les espèces dominantes *Dorymyrmex brunneus* et *Pheidole oxyops* ont été fréquemment observées dans les fragments et les champs de culture indiquant que les fragments peuvent contribuer à une colonisation par les fourmis prédatrices après la récolte ou le travail du sol.

INTRODUCTION

Agricultural production has been intensified during recent decades resulting in large areas of monocultures, and consequently, in a loss of natural and semi-natural areas and in an increased use of agrochemicals (Tschardt et al., 2005 and Kennedy et al., 2013). With the increase of crop fields, semi-natural habitats have also been increasingly fragmented (Tschardt et al., 2012) causing a rapid reduction of species interactions and ecological services (Tylianakis et al., 2008). The resulting loss of biodiversity (Pimm and Raven, 2000) has led to a simplification and homogenization of plant and animal communities, affecting the stability of natural and agricultural ecosystems (McKinney and Lockwood, 1999).

In large-scale monospecific agrosystems, losses in plant diversity have a negative effect on the composition and stability of arthropod populations (Prasifka et al., 2004). Species diversity is heterogeneously distributed in different habitats, landscapes, and regions (Rosenzweig 1995). The factors that allow this heterogeneity are variable and depend on the scale of the analysis (Bohning-Gaese, 1997, Rahbek 2005, Fahr and Kalko, 2011). Tews et al. (2004) found that structurally complex habitats support greater species richness because they provide more ecological niches including available resources (habitat heterogeneity hypothesis).

Thus, the distribution of natural enemies in agricultural landscapes has consequences for biological control services in various agricultural crops. The movement of these insects is fundamental for most organisms and necessary for their survival. In Brazil, sugarcane dominated agricultural landscapes represent such an example for large-scale intensive agriculture with few semi-natural habitats. In our study area (Sao Paulo state), large crop fields are only interrupted by relatively small fragments of the former Atlantic rainforest either along river valleys (gallery forests) or on slopes and plateaus. In addition, the need for larger areas and higher sugarcane production has recently led to the expansion of sugarcane to

former citrus orchards and extensive pasture. Information on the contribution of these forest fragments to the biological control of insect pests in agro-ecosystems is scarce.

Ant species are important natural enemies of insect pests in sugarcane and thus contribute to biological control. Studies of Campiolo (1994) have demonstrated the importance of predatory ants in regulating *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera Crambidae) by egg and larvae predation in sugarcane. Rossi and Fowler (2000) confirmed this finding when reporting that generalist ants contribute to the reduction of sugarcane infestation in the state of São Paulo.

When evaluating the myrmecofauna in sugarcane fields of São Paulo state, Rossi and Fowler (2004) found that *Solenopsis* sp. was the predominant ant genus. In addition, Santos et al. (2017) (in press) showed that the ants *Camponotus* sp., *Crematogaster* sp., *Dorymyrmex* sp. and *Pheidole* sp. were responsible for up to 70% of *D. saccharalis* eggs predation, largely reducing pest population densities. Ant colonies of sugarcane fields do not survive soil tillage and re-colonisation from outside the fields is required to re-establish populations after sugarcane plantation. Semi-natural structures are known to provide resources and other habitat functions for ant species (Offenberg, 2015). Thus it can be assumed that forest fragments in sugarcane dominated landscapes play an important role in such re-colonization processes.

In the present study, we analyzed spatial distribution patterns of predatory ant species along gradients from forest fragments into sugarcane fields. We hypothesized that (1) forest fragments provide shelter and resources after disturbance such as sugarcane harvest during the dry season; (2) consequently predatory ant species are more frequent close to such fragments and their abundance decrease with distance; (3) ant species that colonize sugarcane fields can also be found inside fragments confirming the hypothesis of potential colonization from

fragments into fields; and (4) ant communities differ between river valley and plateau fragments but distance effects are similar (interaction not significant).

MATERIAL AND METHODS

Characterization of the study areas

The study was carried out in sugarcane plantations located near forest fragments in the Jaboticabal municipality of São Paulo state in Southeastern Brazil. The climate is characterized by subtropical-mesothermic, dry winters and a summer rain season, with an annual rainfall of 1,285 mm, a mean temperature of 22°C, and a mean relative humidity of 70.6%. The study area is located in a transition zone between Atlantic rain forest (Mata Atlantica) and the drier savannah systems (Cerrado).

Collections of ants were carried out in twenty different sugarcane fields, nine of which were adjacent to forest fragments of planes and slopes (spontaneous succession but often highly disturbed) and eleven adjacent to gallery forest fragments. Gallery forests are under national protection (Permanent Preservation Areas, PPA) to improve water quality of catchment areas. Afforestation of gallery forests is quite common in the study area resulting in a lack of older trees and adding up to differences in abiotic factors compared with plateau fragments (soil humidity, microclimate).

In each sugarcane field, a transect was set up from inside the forest fragments up to a distance of 100m. Ants were sampled at five different distances along the transect. A field path separating forest fragments and crop was considered as 0 m line. Sampling inside the fragments was performed at a distance of 10 m from this 0 line. Inside the sugarcane field, ants were sampled at a distance of 5 m, 50 m, and 100 m. The distance between areas was at least 500 m. Sampling was carried out using bait traps that specifically attract carnivorous and omnivorous but not herbivorous ants (Fonseca and Diehl, 2004). As a bait trap we used five

grams of canned sardines that were exposed on paper napkin (20 x 20cm). At each distance, ten bait traps were placed every 10 meters along a line parallel to the field edge resulting in a total of 50 traps per transect.

After 30 min exposure, baits and attracted ants were put into plastic bags and transferred to the lab. Ants were sorted by species and sorted samples were stored in 80% alcohol before final identification. Sampled ants were usually identified to species but at least to genus level.

Ants were sampled twice: one month after sugarcane harvest at the end of the dry period (October 2014), and four months later during the rainy season (January 2015).

Statistical analysis

Linear models including distance as continuous factor and fragment type as a categorical factor were tested. Both factors were considered as fixed. A repeated measures design was applied using date as repeated measures factor and including all factor x date interactions. Normality and homoscedasticity were tested before analysis. Analyses were performed using PROC MIXED (SAS Institute, Cary, NC, USA). In the case of significant interactions between distance and the two fixed factors, separate regressions were calculated for date and fragment type to analyze the relationship between distance and ant diversity or abundance.

Multivariate statistics were used to compare predatory ant communities along transects and between fragment types. Since gradient length was shorter than 3 standard deviations PCA was preferred over CA (Smilauer and Leps 2015). Multivariate analysis was performed using Statistica 13 (Statsoft).

RESULTS

In total, 41 ant species belonging to 14 genera were collected (Table 1). The samples taken in October 2014 contained 25 species of ants whereas those taken in January presented 33 species. Eighteen species were found at both dates; seven and 15 species were specific to the October and January sampling, respectively. The most diverse genera was *Pheidole*, with 18 species (49.9% of the total), followed by *Camponotus* with 6 species (14.6%). Five species were common to all areas: *Crematogaster quadriformis*, *Dorymyrmex brunneus*, *Pheidole oxyops*, *Pheidole radoskowskii* and *Pseudomyrmex termitarius*. When comparing the seasons, we found seven species that occurred only after sugarcane harvest during the dry period whereas 12 occurred only three months later (Table 1). After harvest, we found seven only in forest fragments and three species only in sugarcane fields whereas 15 species were common to both habitats. Three months later, three species were exclusive to the fragment areas and eight species were exclusive to sugarcane whereas 21 species occurred in both habitats (Table 1).

The species richness of ants was negatively related to the distance from the forest fragments (Table 2, Fig. 2). However, the significant distance x fragment type x date interaction indicated that the distance effect depends on season and fragment type. The distance effect was stronger after the harvest (dry season) than three months later and this was particularly evident in the plateau fragments. However, the species richness – distance relationship showed a unimodal pattern at the first sampling date in river valleys. Species richness was generally higher close to river valley fragments than close to plateau fragments. The harvest date main effect was significant with a lower species richness after sugarcane harvest.

Table 1. Presence (1) and absence (0) of ant species captured in forest fragments and sugarcane fields one month after harvest (dry season) and three months later (rain season).

Ants species	Dry season				Rainy season			
	Plateau fragment	Sugarcane (Plateau fragment)	River valley fragment	Sugarcane (River valley fragment)	Plateau fragment	Sugarcane (Plateau fragment)	River valley fragment	Sugarcane field (River valley fragment)
<i>Acromyrmex rochai</i>	0	0	0	0	0	0	*	*
<i>Brachymyrmex adnotus</i>	1	1	1	1	0	1	1	1
<i>Camponotus lespesii</i>	0	0	0	0	1	1	0	0
<i>Camponotus</i> sp.	1	0	1	0	0	1	1	0
<i>Camponotus</i> sp.11	*	*	0	0	0	0	0	0
<i>Camponotus crassus</i>	1	1	1	0	1	1	1	1
<i>Camponotus senex</i>	0	0	0	0	1	1	0	0
<i>Camponotus rufipes</i>	0	0	1	0	0	1	0	0
<i>Cephalotes pusillus</i>	0	0	1	0	0	0	0	0
<i>Crematogaster arata</i>	0	0	0	0	0	1	1	0
<i>Crematogaster quadriformis</i>	1	1	1	1	1	1	1	1
<i>Crematogaster</i> sp.7	0	0	0	0	0	1	0	1
<i>Dorymyrmex brunneus</i>	1	1	1	1	1	1	1	1
<i>Ectatomma brunneum</i>	0	0	0	0	0	1	1	0
<i>Ectatomma edentatum</i>	0	0	0	0	0	0	*	*
<i>Gnamptogenys striatula</i>	0	0	0	0	0	0	0	1
<i>Linepithema leucomelas</i>	0	0	0	0	1	0	0	0
<i>Linepithema neotropicum</i>	0	0	0	1	0	0	0	0
<i>Nylanderia</i> sp.	0	0	0	0	1	0	0	0
<i>Odontomachus bauri</i>	0	0	0	0	0	1	0	1
<i>Odontomachus meinerti</i>	0	0	1	0	0	0	0	0

<i>Pheidole cf dione</i>	1	1	0	0	0	0	0	0
<i>Pheidole gertrudae</i>	1	1	0	1	1	1	1	1
<i>Pheidole oxyops</i>	1	1	1	1	1	1	1	1
<i>Pheidole radoskowskii</i>	1	1	1	1	1	1	1	1
<i>Pheidole subarmata</i>	0	0	0	1	1	1	0	1
<i>Pheidole sospes</i>	0	0	1	0	1	0	1	0
<i>Pheidole triconstricta</i>	0	0	0	0	0	1	0	1
<i>Pheidole sp.</i>	1	1	0	1	0	0	0	0
<i>Pheidole sp.9</i>	0	0	0	0	1	0	0	0
<i>Pheidole sp.17</i>	0	0	1	0	0	0	0	0
<i>Pheidole sp.19</i>	1	1	1	0	0	0	0	0
<i>Pheidole sp.36</i>	1	1	0	1	1	1	0	0
<i>Pheidole sp.38</i>	0	0	0	0	0	1	1	0
<i>Pheidole sp.39</i>	0	0	0	0	0	1	0	0
<i>Pheidole sp.40</i>	1	0	0	0	1	1	1	1
<i>Pheidole sp.42</i>	1	1	1	1	0	1	1	1
<i>Pheidole sp.43</i>	0	1	0	0	0	1	0	0
<i>Pseudomyrmex termitarius</i>	1	1	1	1	1	1	1	1
<i>Solenopsis saevissima</i>	0	1	0	0	0	1	0	0

* Indicates that the species occurred only between the fragment and the sugarcane field (0 m) and was not considered in the analysis

The frequency of ants also decreased with distance from forest fragments but the effect was only marginally significant (Figure 3). Interactions with time and fragment type were not significant indicating that distance effects on abundance are more stable than those on species richness. Again, the date effect was significant with a much higher abundance in four months after harvest whereas fragment type did not influence overall ant abundance.

Table 2. Results of mixed model ANOVA on ant species richness and frequency (total and dominant species)

EFFECT	DF	Total Richness		Total Frequency		<i>Dorymyrmex Brunneus</i>		<i>Pheidole oxyops</i>	
		F	P	F	P	F	P	F	P
Fragment type	1	6.19	0.0229	0.14	0.7098	0.46	0.5080	0.97	0.3382
Distance	4	3.09	0.0174	2.02	0.0935	4.32	0.0024	4.94	0.0009
Distance* Fragment	4	0.47	0.7574	0.52	0.7232	1.02	0.4006	1.44	0.2239
Date	1	16.63	<.0001	11.06	0.0011	22.74	<.0001	0.10	0.7517
Date* Fragment	1	0.23	0.6345	0.69	0.4081	1.41	0.2367	0.16	0.6878
Distance*Date	4	1.07	0.3737	1.25	0.2900	1.60	0.1763	0.91	0.4597
Distance*Date* Fragment	4	2.49	0.0450	125	0.2936	1.09	0.3634	0.37	0.8317

Dorymyrmex brunneus Forel, 1908 and *Pheidole oxyops*, Forel, 1908 were the most abundant species (Table 1). The distance effect was significant in both species explained by a decrease in frequency species with distance from forest fragments. The interactions with harvest date and fragment type were not significant confirming the consistent effect of distance effects on the dominant species. *Dorymyrmex brunneus* showed a higher abundance at the second sampling date whereas differences between dates were not significant for *P. oxyops*.

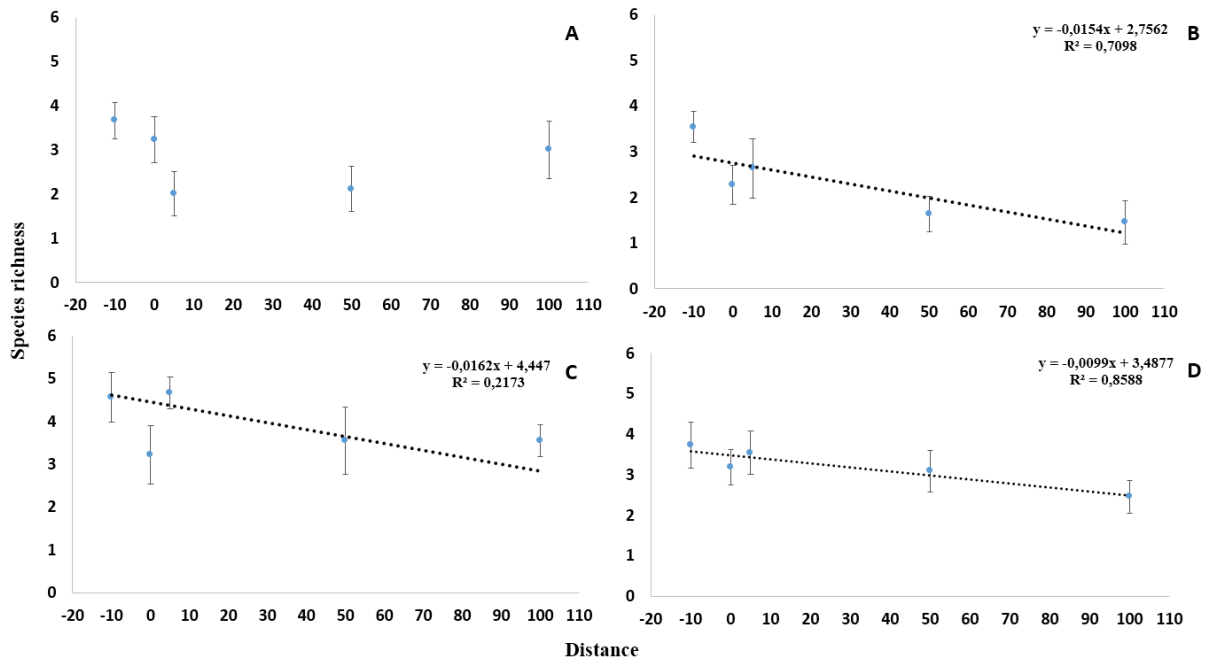


Figure 2. Ant species richness one month (upper: A, B) and four months (lower: C, D) after harvest at different distances from river valley (left: A,C) and plateau fragments (right: B,D). Regression lines are provided for significant relationships. Mean values \pm SE.

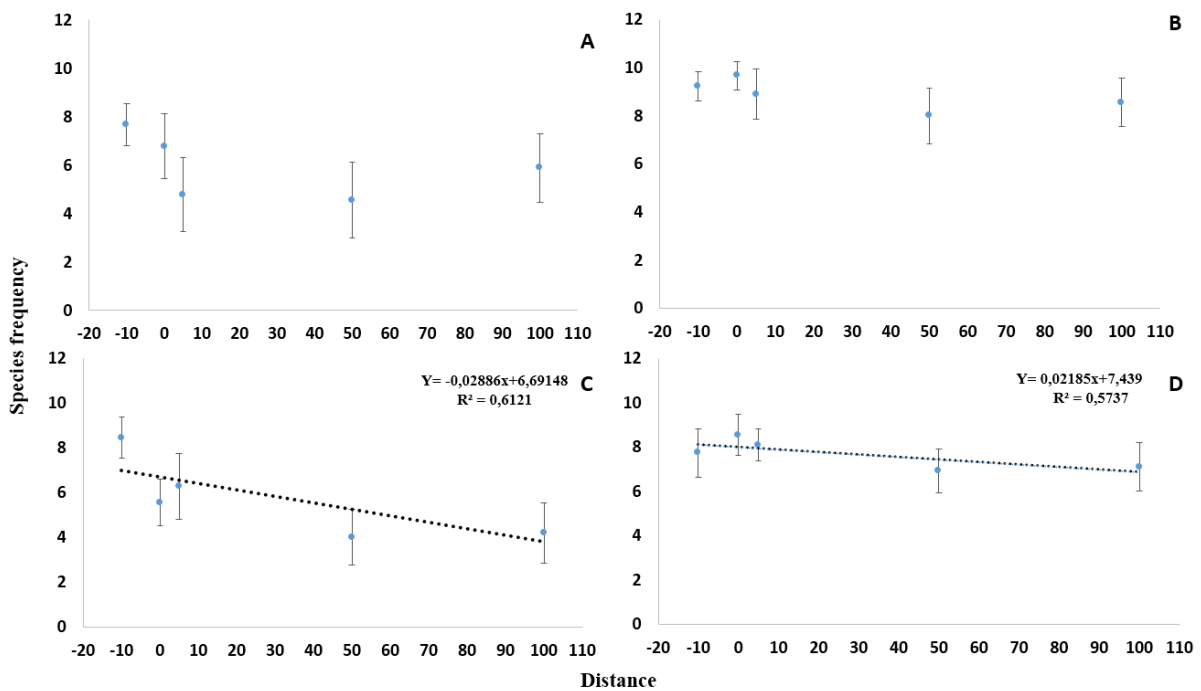


Figure 3. Ant species frequency one month (left: A, C) and four months (right: B, D) after harvest at different distances from river valley (upper graphs: A, B) and plateau fragments (lower graphs: C, D). Regression lines are provided for significant relationships. Mean values \pm SE.

The PCA biplots show that at the first date (Fig. 4 A) ant communities are stronger separated between forest fragments and sugarcane fields than at the second sampling date (Fig. 4 B). One month after harvest, forest fragment samples are concentrated in the upper part and sugarcane samples in the lower part of the biplots. Four months after harvest, several sugarcane samples can also be found in the upper part and several forest fragment samples in the lower part suggesting a migration of ant species that were strongly related to one of the habitats at the first date. The two forest fragment types were not clearly separated neither during the dry season nor during the rainy season indicating a large overlap in ant species composition.

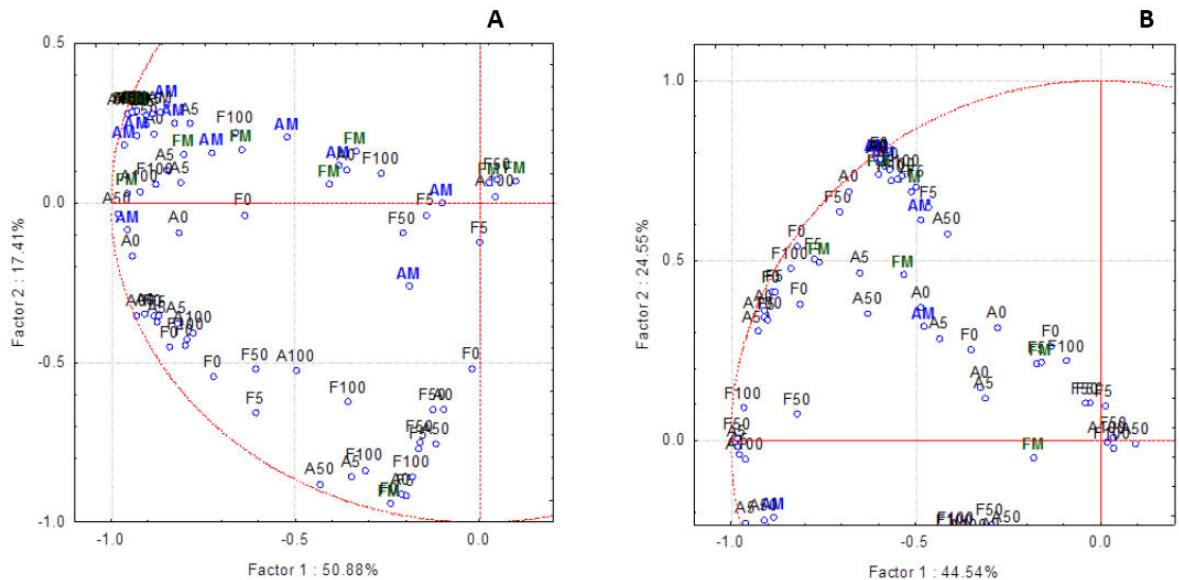


Figure 4. Multivariate analyses of predatory ants communities one month (dry season) (A) and four months (rainy season) (B) after sugarcane harvest. AM (inside river valley fragments, blue); FM inside plateau fragments, green); A0, A5, A50, A100 (sugarcane field at different distances from river valley fragments), F0, F5, F50, F100 sugarcane field at different distances from plateau fragments)

DISCUSSION

We found a decrease in ant species richness and abundance with increasing distance from forest fragments. Forest fragments comprise a higher variety of plant species providing floral and extra-floral nectar, litter and branches, which might strongly affect the

diversity of an ant community (Dejean et al., 2008). Thus, greater availability of space, heterogeneity, food and nesting resources, may have led to a higher amount of ant species in these habitats (Mezger and Pfeiffer, 2011). Vegetation structure of agroecosystems is less diverse not allowing a high diversity of ants species (Offenberg 2015). In addition, the presence of trees may provide greater soil coverage, protection against high temperatures and lower humidity variation favouring ant species (Ribas et al., 2003; Ribeiro and Schoederer, 2007; Gomes et al., 2010). These habitat features may influence dominance structure of ant communities and allow the existence of higher ant species numbers (Perfecto and Vandermeer 1994, 1996).

The species *C. quadrimformis*, *D. brunneus*, *P. oxyops*, *P. radoskowski* and *P. termitarius* were in frequent forest fragments and sugarcane fields. Among them, *D. brunneus* and *P. oxyops* were particularly dominant in sugarcane fields. Agroecosystems are intensively managed limiting decomposing trunks or trees, potential nesting sites and therefore ant diversity (Armbrecht et al., 2004; Foster, 2006). In addition, the competitiveness of these two ant species may further limit ant species richness in crops (Holldobler and Wilson, 1990; Andersen, 2000). The low availability of resources in crop fields increases interspecific competition and competitive exclusion. Apart from reduced competition, increasing resource availability may positively affect the number of individuals (or colonies) in a given area, increasing the number of species just by chance (Ribas et al., 2003). *Dorymyrmex* species are very competitive in disturbed (Hölldobler and Wilson 1990, Andersen 1997, Majer and Nichols 1998) and open (Cuezzo and Guerrero 2012) habitats, which explains their high abundance in crop fields compared with forest remnants. *D. brunneus* is abundant in sugarcane fields (Souza et al., 2010) and is an important predator of sugarcane borer *Diatraea saccharalis*.

The relatively high overlap of ant species captured in forest fragments and in sugarcane fields demonstrated that these natural habitats are potential refuges of ant species regulating pest insects in sugarcane fields during unfavorable environmental periods such as harvest or soil tillage. Re-colonization from forest fragment refuges into the fields was confirmed by the observation that differences between fragments and sugarcane fields (species richness and to a lesser degree ant frequency) were larger in October after disturbance by sugarcane harvest than later in the growing season suggesting a re-colonization starting from these natural habitats. Additionally, PCA clearly separated ant communities of sugarcane field after disturbance by sugarcane harvest whereas later in the season differences were less strong. In the post-harvest period, the number of species limited to one of the habitats was higher in fragments, whereas four months after the harvest this number was higher in sugarcane fields. Re-colonization may have been the result of an increase in resource availability (shelter, food, and protection) with the growth of sugarcane plants. Plant diversity (weeds) and litter cover increasing with crop growth may have favored the species richness of the ant community (Silva et al., 2011). Different resources are required for foraging and nesting, which is essential for the diversity of ants (Fowler et al., 1991, Vasconcelos 2008, Silva et al., 2011, Saad et al. 2017).

However, observation dates do not only refer to time since major disturbance by harvest. Although smaller than in sugarcane fields, differences between observation dates were also found in the forest fragments not suffering from disturbance. This may be due to the greater availability of food resources during the rainy season in January. In our study, we cannot separate effects of disturbance by harvest or of humidity. However, the stronger differences inside sugarcane fields indicate that the observation date effect is at least partly related to crop management.

Plateau fragments showed a higher richness of ant species when compared to river valley fragments. The first fragment type may have provided more resources for ant species, such as shelter and food availability (Tews et al., 2004; Stein et al., 2004). River valley fragments (Permanent Preserved Areas) are commonly found near rivers of the study region and the higher humidity increases productivity.

Our study showed the importance of forest fragments as (semi-)natural habitats for the richness and abundance of predatory and omnivorous ant species. Since several species and species groups are involved in the control of sugarcane pest insects such as the sugarcane borer these habitats may play a major role in natural pest insect regulation. The higher overlap of sugarcane and forest fragment ant communities several months after sugarcane harvest suggest a partial post-harvest recolonization of sugarcane fields from forest fragments. Although different in species richness and abundance levels, the ant abundance/species richness – distance relationships are similar for the two major forest fragment type, river valley and plateau fragments.

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CHAPTER III- INFLUENCE OF ORCHARD MARGIN VEGETATION ON THE CONTROL OF ROSY APPLE APHID *DYSAPHIS PLANTAGINEA* AND ON THE SPATIO-TEMPORAL DISTRIBUTION OF NATURAL ENEMIES

RESUMO

O pulgão rosado da maçã *Dysaphis plantaginea* é um importante inseto praga nos pomares de maçã, pois causam perdas econômicas elevadas. O uso do controle biológico de conservação utilizando espécies de plantas que fornecem recursos para inimigos naturais pode melhorar a regulação natural e reduzir o uso de inseticidas. Neste estudo, foi comparado a influência de faixas de flores silvestres, faixas de grama e vegetação espontânea sobre o controle biológico de pulgões por seus inimigos naturais. A mistura de faixas de flores silvestres semeadas foi bem sucedida no aumento de recursos florais em ambos os anos. Entre os dois principais grupos inimigos naturais observados nas faixas de margem, apenas os sirfídeos responderam positivamente a este aumento de recursos, enquanto a abundância de coccinelídeos não foi significativamente diferente entre os tratamentos de faixas. Dentro dos pomares, nenhum dos três grupos inimigos naturais observados (parasitóides, sirfídeos, coccinelídeos) respondeu aos tratamentos de faixas adjacentes e a predação de pulgões sentinelas não foi significativamente diferente. O número de inimigos naturais observados nas colônias de afídeos foi principalmente impulsionado pelo número de afídeos, embora a regressão linear não tenha sido significativa para o tratamento com faixa de flores silvestres. Os números de pulgões foram geralmente mais altos perto das margens, sugerindo que a colonização de pulgões nas margens do pomar pode neutralizar os efeitos positivos na abundância do inimigo natural. Os resultados confirmam a influência positiva do aumento de recursos florais por faixas de flores silvestres na regulação de afídeos, mas também demonstram que o tamanho

do efeito pode ser pequeno se a vegetação espontânea, rica em espécies já ocorrer nas margens ou dentro dos pomares.

ABSTRACT

The rosy apple aphid *Dysaphis plantaginea* is a major pest insect in apple orchards causing high economic losses. A conservation biological control approach using plant species that provide resources for natural enemies may improve natural regulation and reduce insecticide use. In this study, we compared the influence of wildflower strips, grass strips and spontaneous vegetation on the biological control of aphids by their natural enemies. The sown wildflower strip mixture was successful in increasing floral resource provisioning in both years. Among the two major natural enemy groups observed in the margin strips only hoverflies responded positively to this higher resource provisioning whereas ladybird abundance was not significantly different between strip treatments. Within the orchards, none of the three observed natural enemy groups (parasitoids, hoverflies, ladybirds) responded to the adjacent strip treatments and predation of sentinel aphids was not significantly different. The number of natural enemies observed in aphid colonies was mainly driven by aphid number, although the linear regression was not significant for the wildflower strip treatment. Aphid numbers were generally higher close to the margins suggesting that aphid colonization of orchard edges may counteract positive margin effects on natural enemy abundance. The results confirm the positive influence of floral resource provisioning by wildflower strips on aphid regulation but they also demonstrate that effect size may be small if species-rich spontaneous vegetation does already occur in margins or inside orchards.

RÉSUMÉ

Le puceron rosé de la pomme *Dysaphis plantaginea* est le majeur insecte nuisible dans les vergers de pommiers et causant des pertes économiques élevées. Une approche de contrôle biologique de conservation utilisant des espèces végétales qui fournissent des ressources aux ennemis naturels peut améliorer la régulation naturelle et réduire l'utilisation d'insecticide. Dans cette étude, nous avons comparé l'influence des bandes fleuries sauvages, des bandes d'herbe et de la végétation spontanée sur le contrôle biologique des pucerons par leurs ennemis naturels. Le mélange de bandes de fleuries sauvages semées a réussi à augmenter le provisionnement des ressources florales au cours des deux années. Parmi les deux principaux groupes d'ennemis naturels observés dans les bandes de marges, seuls les hoverflies ont répondu positivement à ce renforcement des ressources tandis que l'abondance des coccinelles n'était pas significativement différente entre les traitements à la bande. Dans les vergers, aucun des trois groupes d'ennemis naturels observés (parasitoïdes, hoverflies, coccinelles) n'a répondu aux traitements de bande adjacents et la prédation des pucerons sentinelles n'était pas significativement différente. Le nombre d'ennemis naturels observés dans les colonies de pucerons était principalement déterminé par le nombre de pucerons, bien que la régression linéaire n'ait pas été significative pour le traitement de la bande fleuries. Les nombres de pucerons étaient généralement plus élevés près des marges, ce qui laisse supposer que la colonisation des pucerons par les pucerons peut contrecarrer les effets positifs de la marge sur l'abondance naturelle de l'ennemi. Les résultats confirment l'influence positive de l'approvisionnement en ressources florales par les bandes fleuries sur la régulation des pucerons, mais ils démontrent également que la taille de l'effet peut être faible si la végétation spontanée riche en espèces se produit déjà dans les marges ou dans les vergers.

INTRODUCTION

The rosy apple aphid, *Dysaphis plantaginea* Passerini (Hemiptera: Aphididae) (RAA), is a major pest insect in apple orchards causing considerable damage, in particular a deformation of fruits (Cockfield et al., 2012). Due to the economic losses and the socio-economic pressure to reduce pesticide applications, an increasing number of studies analyzed the efficiency of naturally occurring parasitoids and predators in orchards to control RAA populations (e.g. Dib et al., 2010). Conservation biological control based on the manipulation of non-crop habitats has been discussed to support or replace traditional biocontrol approaches based on natural enemy release (Jonsson et al., 2008; Griffith et al., 2008; Balzan et al., 2014). An appropriate management of such semi-natural structures in the surrounding landscape improves resource provisioning and other habitat functions of natural enemies (Landis et al., 2000).

Several habitat functions depend on their plant species composition. Plant species provide shelter, alternative prey and overwintering places for arthropods (Landis et al., 2000; Griffiths et al., 2008). Adult parasitoids and hoverflies need nectar or pollen to complete their life cycles (Wackers, 2004; Laubertie et al. 2012). The sugar provided in floral or extrafloral nectar increases the longevity of the natural enemies (Lavandero et al., 2006; Winkler et al., 2009; Laubertie et al. 2012) and the efficiency of pest control (Winkler et al., 2006).

Therefore, wildflower strips optimizing floral resources and other habitat functions have been suggested to improve conservation biological control (Griffiths et al., 2008; Pfiffner et al., 2009; Tschumi et al., 2015). A positive effect of such wildflower strips on natural enemy abundance and associated ecosystem services has been demonstrated in several studies (Haaland et al., 2011). The choice of plant species for wildflower strips need to take into account the amount and quality of floral and extrafloral nectar, the floral morphology, and the feeding preference of natural enemies (Sivinski et al., 2011; Campbell et al., 2012;

Wäckers et al., 2012). Plants in field margins may also provide resources for pest insects and thus increase crop damage (Koji et al., 2007). So, plant species in wildflower strips need to be properly selected to favour natural enemies without increasing pest abundance (Lee and Heimpel, 2005).

The RAA is attacked by numerous generalist predator species such as spiders and earwigs (Dib et al., 2011; Boreau de Roince et al., 2013) and aphidophagous specialists such as hoverflies, ladybirds, and hymenopteran parasitoids (Wyss et al., 1995, 1999; Dib et al., 2010, 2012). Hoverflies, hymenopteran parasitoids and earwigs were the most efficient natural enemies of the RAA in south eastern France (Dib et al., 2010, 2011). The most frequently observed species among these taxa were the hoverfly *Episyrphus balteatus* (Syrphidae), the parasitoid *Ephedrus persicae* (Braconidae) and the earwig *Forficula auricularia* (Forficulidae) (Dib et al., 2010). Hoverflies and parasitoids depend on nectar and pollen and it was hypothesized that vegetation providing these resources may improve aphid regulation in apple orchards (Marko et al., 2013; Miñarro and Prida, 2013).

We designed a wildflower strip mixture comprising flowering species with different functional traits and life cycles (early to late flowering, annual to perennial, and various floral morphologies) in order to optimize floral resource provisioning for high quality, quantity and duration. The effects of this wildflower strip mixture were compared with two different controls, spontaneous vegetation and species-poor grass strips. The impact of wildflower strips on the dynamics of the arthropod communities have usually been tested against a complete absence of non-crop vegetation (Pfiffner et al., 2009; Haaland et al., 2011; Tschumi et al., 2016). However, spontaneous vegetation may be as efficient as flower strips in attracting pest natural enemies (Denys and Tschardtke, 2002) without producing costs for seed material and strip management. Implementations of grass strips have also been tested as a cost efficient way to improve pest insect regulation (Collins et al., 2002; Al Hassan et al.,

2013). They provide less floral resources but generalist predators such as ground beetles, rove beetles, and spiders may benefit from their shelter function (Wyss et al., 1995; Collins et al., 2002).

An efficient control of RAA requires that its natural enemies move from the field margins into the apple orchard. Most studies on wildflower strips are limited to observations of natural enemy diversity and abundance within the strips (Haaland et al., 2011; Blaauw and Isaacs 2014). The studies that analyzed the presence of natural enemies both in wildflower strips and within the crops usually confirmed the attraction of natural enemies by flowers, but without a corresponding reduction of insect pests (Pfiffner and Wyss 2004; Haaland et al., 2011). The few studies demonstrating a significant contribution of wildflower strips to pest control, showed a reduction of regulatory effects with the distance to field margins (Collins et al., 2002; Tylisanakis et al., 2004; Tschumi et al., 2015). A better understanding of the spatial dynamics of natural enemies is needed to evaluate the efficiency of wildflower strips in regulating aphids in orchards.

In this study, we analyzed the influence of three margin strips (wildflower strips, grass strips, and spontaneous vegetation) on the abundance of RAA and its natural enemies from the margin into apple orchard in order to test the following hypotheses: (I) sowing of wildflower strips optimized for nectar and pollen production increases natural enemy abundance in orchard margins in comparison with grass and spontaneous vegetation strips; (II) the attraction of natural enemies by flowering plant species in the margins also increases their abundances inside the orchards; (III) due to higher natural enemy density the abundance of RAA decreases close to orchard margins presenting floral resources; (IV) orchard margin effects on natural enemies abundances and aphid control decrease with distance to the edge.

MATERIAL AND METHODS

Study sites and design

The experiment was set up in three apple orchards at INRA Saint-Paul, Montfavet, France (43°54'51.57"N, 4°52'56.15"E) and run from March 2014 until June 2015. The orchards comprised either five rows of 24 apple trees or six rows of 48 apple trees. In March 2014, a 2.5 meter-wide strip was manually sown at the north or the south margin of each orchard (Fig. 1). To prepare the seed bed, each strip was previously ploughed, then harrowed to remove weed seedlings emerged after ploughing. Three strip treatments were established in each orchard: (a) a wildflower strip mixture comprising 30 vascular plant species (WS) optimized for a high and long lasting production of floral resources, (b) a grass strip mixture (GS) including two perennial grasses, *Lolium perenne* (28%) and *Festuca arundinacea* (72%), recommended in France to limit pollution of water bodies by fertilizer and pesticide contamination, (c) an unsown strip allowing the natural development of spontaneous vegetation (SV). The position of the strip treatments at the orchard edge was randomized within each orchard margins and thus each orchard represented a replicate block (Fig. 1). No insecticides were applied during the study period.

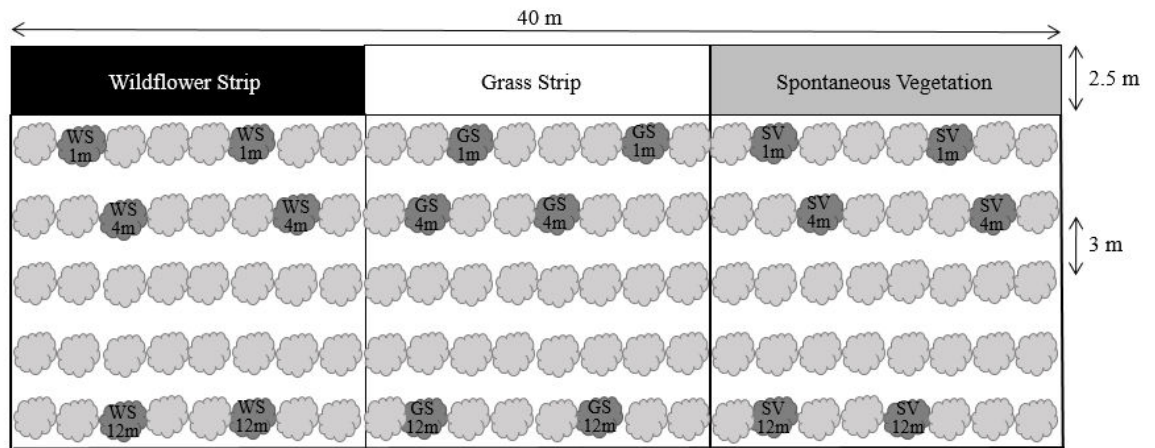


Fig. 1. Experimental design. Shaded symbols indicate observation trees at 3 distance classes (1 m, 4 m and 12 m).

Observations within margin strips

In order to evaluate differences in plant species composition and provisioning of floral resources, the cover of all vascular plant species was recorded in spring of 2014 and 2015. At the same time, phenology of these plants was analyzed by observing the percentage of vegetative, flowering and fruiting plants. As a proxy of floral resource provisioning we used the percentage of flowering entomophilous plants. We considered a species as entomophilous if indicated as insect-pollinated in the BiolFlor database (Kühn, et al. 2004). The total cover of entomophilous species was multiplied by the estimated proportion of flowering individuals at the respective survey date to estimate the cover of flowering entomophilous plants. Survey dates were 6 June 2014 and the 22 May 2015 corresponding to peak abundance of RAA.

At the same dates, aphidophagous predators (hoverfly and ladybug adults) that were visible from outside the strip were counted during 10 min in each margin strip. The presence of larvae of these two aphid predators was directly observed on the plants within the margin strips. Both counts were added to estimate the abundance of hoverfly and ladybug predators

per margin strip. The entomological observations were limited to a period between 10:00 and 17:00 under favorable weather conditions (sunny, no rain, low wind speed).

Observations within apple orchards

Depending on orchard size, within-field observations were performed on 2 or 3 labeled trees per treatment and distance resulting in a total of 18 or 27 analyzed trees per orchard. According to the position of the apple tree rows, the distances of the observation trees were 1, 4, and 12 m from the margin strips (Fig. 1). All observations were performed on the same labeled apple trees. Observations included the number of RAA colonies, the presence of natural enemies in these aphid colonies, and the predation of sentinel aphids detailed below. Terminal growing shoots were counted to take into account apple tree vigor because only those shoots are attacked by RAA (Simon et al., 2012). All observations were made twice a year at the end of April or the beginning of May (session 1) and four weeks later (session 2).

Aphid infestation was estimated as the proportion of terminal growing shoots with a *D. plantaginae* colony per apple tree. Three RAA colonies were randomly chosen on the northern and the southern sides of each observation tree to record the presence of aphid mummies (dead bodies of aphids parasitized by braconid wasp) and of natural enemies (mainly hoverflies, ladybugs, and earwigs) according to Dib et al. (2016).

Finally, sentinel aphids glued to sandpaper cards were used to evaluate aphid predation (Östman, 2004). Three living aphids were attached to each 5 cm x 5 cm sandpaper card. The cards were fixed at the abaxial leaf surface of the observation trees (1.5 m above the ground) and were removed the following day. Aphid predation was estimated as the proportion of sentinel aphids per card attacked during 24 h.

Statistical analysis

We used generalized linear models (*glmer* function, lme4 package, R version 3.2.4) to test: (I) the effect of the strip treatment on the number of vascular plant species, on the cover of flowering entomophilous plants, and on the abundance of natural aphid enemies in the orchard margins; (II) the effects of the strip treatment, of the distance to the orchard margin and the treatment x distance interaction on the abundance of RAA colonies per apple tree (aphid infestation and aphid colony increment), on the presence of natural enemies per RAA colony and apple tree, and on the predation of sentinel aphids; (III) correlations between the number of aphids and the number of aphid natural enemies within RAA colonies. To take into account for temporal variation in the observed ecological data, year, and observation date (when relevant) were fitted as additional factors. Orchard was included as a block factor in all the models.

The number of vascular plant species and the cover of flowering entomophilous plants ($\arcsin[\sqrt{\%}]$ transformed) were fitted using a Gaussian error distribution with identity link function. The abundance of natural enemies within margin strips was analyzed using a Poisson error distribution with log link function. Finally, the binary data of aphid infestation, aphid colony increment, predation of sentinel aphids and presence of mummies and/or predators in a RAA colony were analyzed using a binomial error distribution with a logit function. Linear regressions were calculated to analyze relationships between the number of aphids (ln-transformed) and number of natural enemies (ln-transformed) within the RAA colonies.

Strip treatment (WS, GS, SV), distance to the margin strips (1, 4, and 12 m), year (2014 and 2015) and observation dates within year (sessions 1 and 2) were fitted as fixed factors and orchard was included as a random factor in each model. In the case of over-dispersion, observation tree was included as an additional random factor (Harrison, 2014).

The *Anova* and *glht* functions (car and multcomp R libraries, respectively) were used to test factors and their interactions (Wald's sequential probability likelihood ratio tests) and to compare the effects of a particular strip treatment and distance classes within each significant factor (Z-tests).

RESULTS

Observations within margin strips

The cover of flowering entomophilous species as a proxy of floral resource provisioning was in both years significantly higher (2.5 fold on average) in wildflower strips than in grass strips and in spontaneous vegetation (Fig.2 A, B). However, in 2014 the difference was only marginally significant between the wildflower and spontaneous vegetation strips ($|Z|=2.3$, $P=0.059$). No significant difference occurred between the spontaneous vegetation and grass strips. In 2014, the sown species represented only 25% of the entomophilous species cover in wildflower strips, whereas their proportion was 90% in 2015. Plant diversity as number of vascular species per strip was also significantly higher in wildflower strips than that in grassy and spontaneous vegetation strips (Fig 2 C, D). On average wildflower strips presented ten more plant species than the other treatments. In 2015, the number of plant diversity was lower in the grassy than in the spontaneous vegetation strips whereas no such a difference was found in 2014.

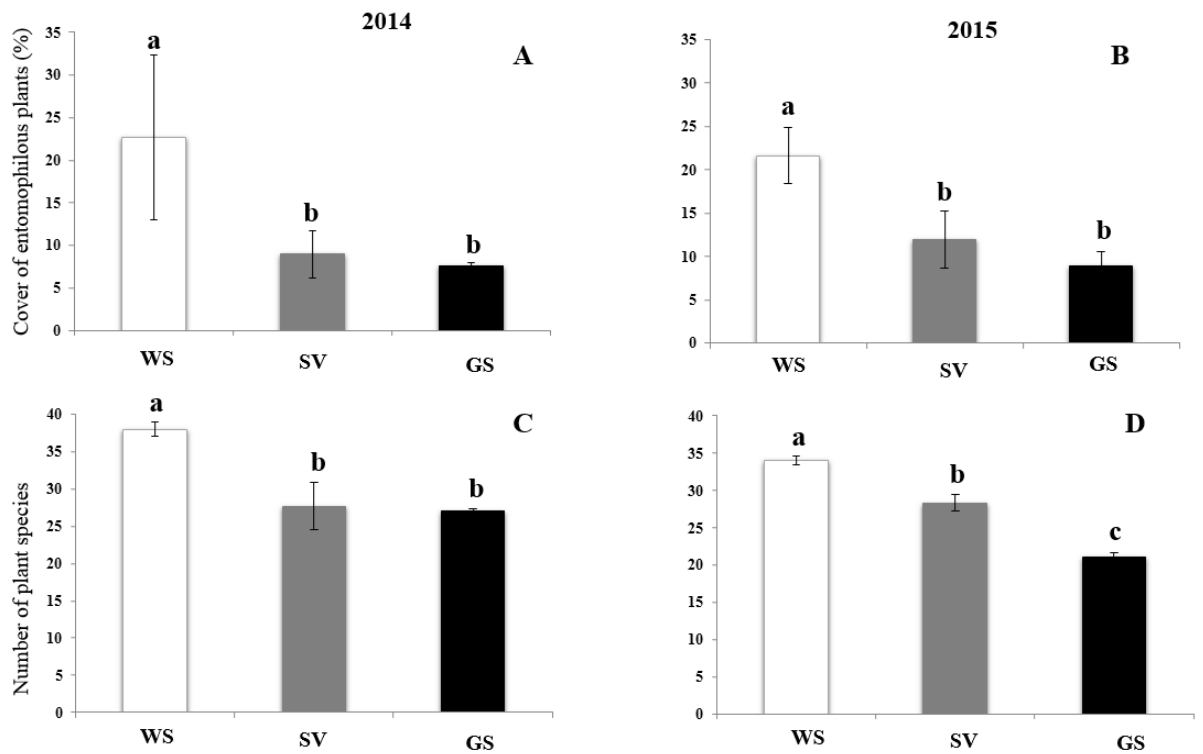


Fig.2. Mean (\pm SE) cover of entomophilous flowering plants (A, B) and mean number of plant species (C, D) of three different field margin types in 2014 (A, C) and 2015 (B, D); WS: wildflower strip, SV: spontaneous vegetation strips; GS: grass strip. Different lowercase letters above columns indicate significant differences at $p < 0.05$.

Hoverflies and ladybugs were the most abundant aphid predators recorded in the margin strips. Hoverfly abundance differed significantly between years ($X^2=50.2$, $P=1.3 \times 10^{-12}$) and strip treatments ($X^2=11.8$, $P=0.003$). Hoverfly abundance increased from three individuals per strip on average in 2014 to twelve individuals per strip on average in 2015. The significant treatment strip treatment effect was explained by a higher hoverfly abundance in the wildflower strips compared with grassy and the spontaneous vegetation strips (in 2014: $|Z| < 0.5$, $P > 0.62$; in 2015: $|Z| > 2.9$, $P < 0.008$; Fig 3 A, B). Ladybug abundance within margin

strips (mean= 3.4 ± sd 4.4) did neither differ between years ($X^2=0.82$, $P=0.36$) nor between treatments ($X^2=0.71$, $P=0.70$).

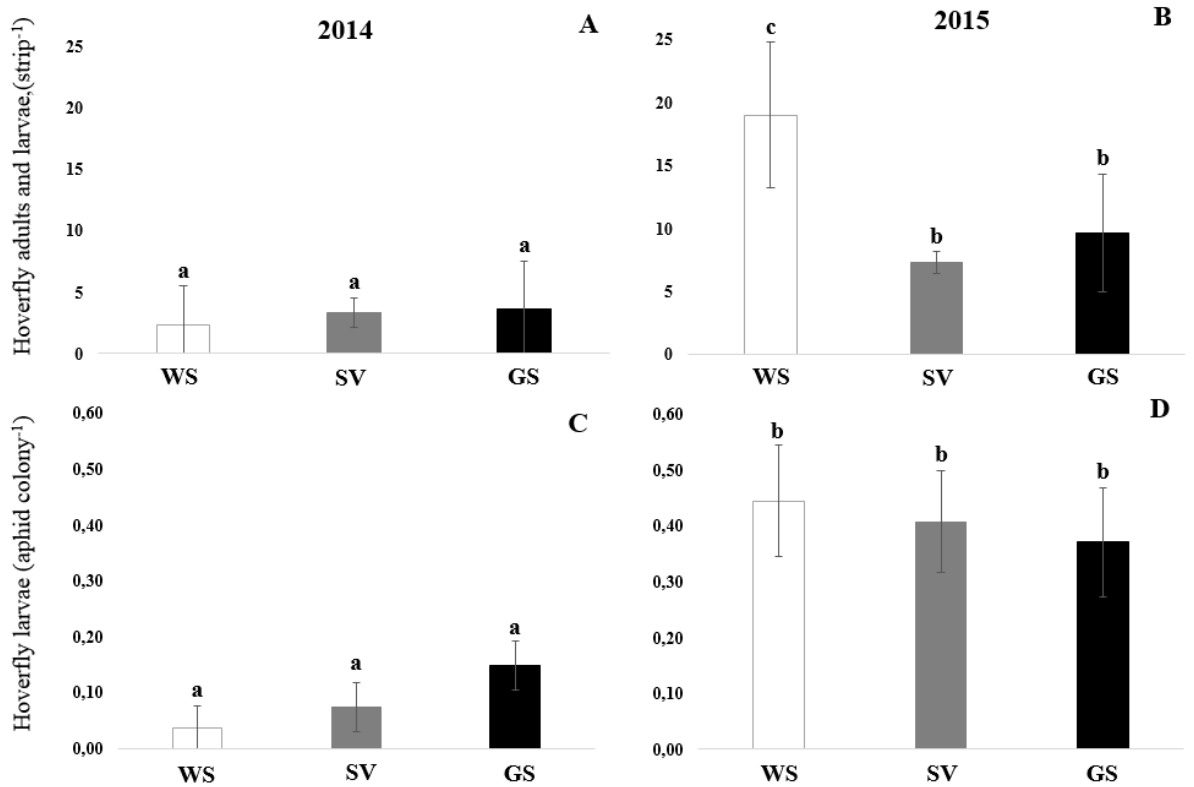


Fig. 3. Mean (\pm SE) number of hoverflies observed in margin strips (A and B) and in *D. plantaginae* colonies inside the orchards (C and D) in 2014 (A, C) and in 2015 (B, D); WS: wildflower strip; SV: spontaneous vegetation strips; GS: grass strip. Different lowercase letters above columns indicate significant differences at $P < 0.05$.

Observations within apple orchards

Inside orchards, ladybugs, hoverflies, and earwigs were the most frequently observed predators within the RAA colonies (Table 1). Aphid mummies were only observed at the second spring session. Natural enemy occurrence on observation trees of all these RAA natural enemies differed significantly between years ($X^2=15.4$, $P=8.3 \times 10^{-5}$) and between

spring sessions ($X^2=65.4$, $P=5.8 \times 10^{-16}$), but not between margin strip treatments ($X^2=1.5$, $P=0.46$), or between distance classes to the margin strip ($X^2=0.6$, $P=0.74$). The occurrence of hoverfly larvae doubled between 2014 and 2015 (25% to 54 % of the apple trees; $X^2=13.7$, $P=2.1 \times 10^{-4}$). However, an increase of numbers in the wildflower strip treatment observed in the margins was not confirmed by a higher proportion of hoverflies in aphid colonies inside orchards (Fig 3 C, D). Earwigs were frequently observed in 2014 but almost absent in 2015 (42% to 2% of the apple trees; $X^2=77.8$, $P=2.0 \times 10^{-16}$). The occurrence of aphid mummies was higher in 2014 than in 2015 (difference marginally significant: 75% and 56% of the apple trees, respectively; $X^2=3.5$, $P=0.06$).

Table 1. Mean number of *D. plantaginae* colonies per apple tree (aphid colonies) and mean proportion of aphid colonies attacked by predators (hoverflies, ladybirds and earwigs) or parasitoids (recorded as aphid mummies) in 2014 and 2015. Minimum and maximum values are indicated in brackets.

	2014		2015	
	6 May	3 June	24 April	19 May
Aphid colonies	19.9 [15.3-24.6]	41.9 [31.8-56.0]	1.5 [1.5-1.6]	22.3 [11.3-27.4]
Hoverflies	0.3 [0.2-0.5]	0.1 [0.0-0.4]	0.0 [0.0-0.0]	0.7 [0.4-0.9]
Ladybirds	0.1 [0.0-0.2]	0.6 [0.5-0.9]	0.0 [0.0-0.0]	0.5 [0.2-0.7]
Earwigs	0.1 [0.1-0.2]	0.6 [0.5-0.9]	0.0 [0.0-0.0]	0.2 [0.0-0.8]
Parasitoids	0.0 [0.0-0.0]	0.7 [0.5-1.0]	0.0 [0.0-0.0]	0.6 [0.3-0.7]

On average, 18 RAA colonies per tree and date were observed (Table 1). Aphid infestation was estimated as the proportion of terminal growing shoots carrying a RAA colony. Infestation by RAA was significantly different between years, sessions within years

and distances from the margin strips (Table 2). Infestation by RAA was three times lower in 2015 than in 2014 (16% and 55% of the growing shoot, respectively). The highest infestation by RAA was observed at the closest distances to the orchard margins (36% and 33% of the growing shoots at 1 m and 12 m distances, respectively). Although the strip treatment main effect was not significant, the significant treatment x distance interaction showed that the treatment effect depended on distance from the margin. At a distance of 1 m from the wildflower strips aphid infestation was lower than at the same distance to the other margins (only 33% of the growing shoots), whereas no significant treatment differences were found at 4 m and 12 m.

Table 2. Temporal and spatial variation of aphid infestation (proportion of terminal growing shoots with at least one *D. plantaginae* colony) and aphid colony increment (increase in the number of *D. plantaginae* colonies from session 1 to session 2). Temporal and spatial interactions refer to year x session and to margin strip treatment x distance interactions, respectively.

	Aphid infestation			Colony increment	
	<i>df</i>	X^2	<i>P</i> -value	X^2	<i>P</i> -value
Year	1	102.4	2.2×10^{-16}	0.4	0.548
Session	1	12.3	4.6×10^{-4}	/	/
Temporal interaction	1	21.7	3.2×10^{-6}	/	/
Strip treatment	2	3.1	0.215	0.7	0.704
Distance to margin	2	8.7	0.013	5.1	0.079
Spatial interaction	4	11.2	0.025	5.1	0.279

The increment of the number of RAA colonies between first and second session was measured to evaluate the effect of natural enemies on the level of aphid infestation. The proportion of apple trees showing an increase in the number of RAA colonies was marginally higher at the closest distances to the orchard margins (71% and 61% of the apple trees at 1 m and 12 m distances respectively; Table 2).

On average, 25% of the sentinel aphids were preyed, but their predation was neither spatially structured within the orchards between distance classes ($X^2=0.4$, $P=0.810$), nor margin strip treatments ($X^2=0.2$, $P=0.906$), nor temporally structured between sessions ($X^2=0.2$, $P=0.690$) or year ($X^2=0.1$, $P=0.799$).

Observations within rosy apple aphid colonies

Linear regressions between the number of aphids and the total number of their natural enemies in RAA colony were performed to verify density-dependence effects of margin strip treatments. Numbers of natural enemies per RAA colony were significantly correlated to numbers of aphids (one natural enemy for 34 aphids on average; slope: $X^2=14.93$, $P=1.1 \times 10^{-4}$; intercept: $X^2=5.04$, $P=0.025$). However, separate linear regressions for margin strip treatments were only significant for spontaneous vegetation (slope: $X^2=14.93$, $P=1.1 \times 10^{-4}$) but not for wildflower and grass strips (Fig. 4). Nevertheless, the intercept was only significantly different from 0 in the wildflower strip treatment (intercept: $X^2=4.28$, $P=0.039$; Fig. 4) indicating a higher availability of natural enemies in the absence of aphids.

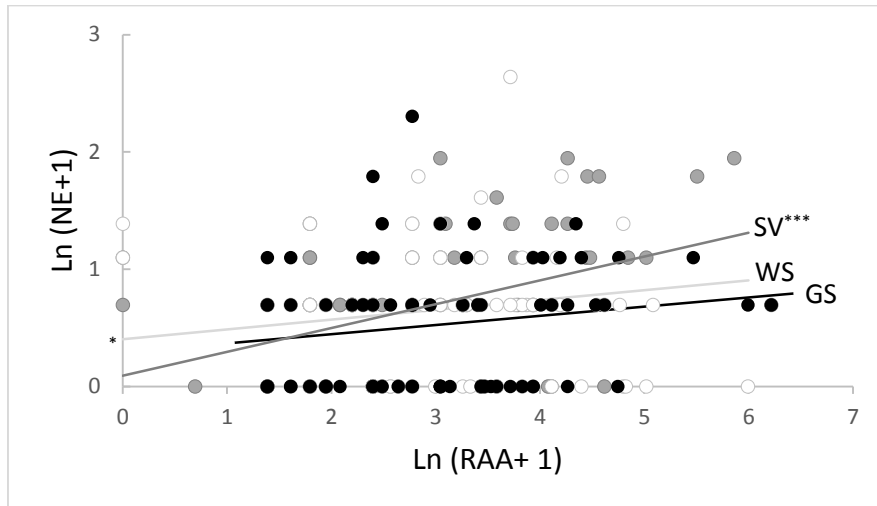


Fig. 4. Relationship between number of aphids (RAA) and number of natural enemies (NE) per aphid colony at second session 2015 (ln-transformed values). Regression lines are presented to illustrate slope and intercept differences between WS (wildflower strip, white circles), SV (spontaneous vegetation strips, grey circles), and GS (grass strip, black circles) treatments. Stars indicate slopes or intercepts that significantly differ from zero (*: $P < 0.05$; ***: $P < 0.001$).

DISCUSSION

Our results show that the approach to increase floral resource provisioning by sowing a plant mixture optimized for quantity and duration of nectar production was successful. The cover of flowering entomophilous plants species as a proxy for nectar and pollen production was significantly higher in wildflower strips than in grass and spontaneous vegetation strips. Plant diversity was also higher although not all sown species developed in the wildflower strips. Only small differences occurred between grassy and spontaneous vegetation strips but the diversity of grass strips was lower in the second year confirming the suppressive effect of competitive grass mixtures on spontaneously emerging species (Cordeau et al., 2012). Spontaneous vegetation emerging from the soil seed bank considerably contributed to both

plant diversity and floral resources in both sown strips reducing the magnitude of treatment effects compared with other studies (Bischoff et al., 2016).

Among the two major aphid predators observed in the strips, only hoverflies responded to the strip treatment. Hoverfly densities were higher in the wildflower strips than in the other strips. Adult hoverflies rely on nectar resources (Wäckers, 2004; Fiedler et al., 2008). Laboratory experiments on the aphidophagous hoverfly *Episyrphus balteatus* showed that adult fitness depends on floral resource provisioning (Laubertie et al., 2012). Marko et al. (2013) and Miñarro and Prida (2013) also found a positive effect of the surrounding vegetation on hoverfly abundance in orchards. In our study, the effect of wildflower strips on hoverfly abundance was only significant in the second year confirming that plant–hoverfly interactions depend on plant species composition (Laubertie et al., 2012; van Rijn and Wäckers 2016). Annual plant species were dominant during the first year but were replaced by biennials and perennials in the course of plant succession.

Ladybirds did not respond to the strip treatments. Although occasionally feeding on pollen and nectar (Triltsch, 1999), their performance depends much less on floral resources because larvae and adults are predominantly predators (Ricci et al., 2005). Nevertheless, several other studies found a positive effect of floral resources on ladybird abundance (Tschumi et al., 2015; Bischoff et al., 2016).

The effect of strip treatments on natural enemies observed in aphid colonies inside orchards was not significant. The energetic costs and the relative attractiveness of margins may have reduced natural enemy movements into the field (Heimpel and Jervis, 2005; Wanner et al., 2006). However, hoverflies that strongly responded to the treatments in the strips are highly mobile and it is not very likely that limited movement was responsible for a failure in detecting treatment effects inside orchards. Hoverflies may show a stronger response to large-scale features reducing the influence of small scale resource provisioning

(Wratten et al., 2003). Additionally, a time lag between higher adult abundance measured in wildflower strips and the occurrence of larvae in the orchards has to be considered. Effects of resource provisioning on adult oviposition may occur later, larvae hatch after a couple of days and larvae feed for about three weeks on aphids (Laubertie et al. 2012, van Rijn and Wäckers 2016).

Similarly to natural enemies observed in apple orchards, the overall treatment effect on aphid abundances was not significant. However, a significant treatment x distance interaction was detected resulting from a lower aphid infestation close to wildflower strips (1 m distance) whereas no such treatment effect was observed at higher distances. Since no corresponding result was obtained for the three natural enemy groups observed in aphid colonies (parasitoids [estimated from mummy number], hoverflies, ladybirds), other predators coming from the wildflower strips may be responsible for these findings. Although floral resources do not necessarily increase ground beetle abundance (Balzan et al. 2014), a positive effect of wildflower strips on this important generalist predator of aphids has been reported (Tschumi et al. 2015). However, we did not find any differences in predation of sentinel aphids glued on predation cards, neither between distances nor between treatments suggesting that this method does not sufficiently account for RAA predation in the orchards.

In general, aphid density and the increment of aphid colony number during spring were higher close to the margin strips than inside the orchards. Ribwort, *Plantago lanceolata*, is the RAA secondary host during summer (Dib et al. 2011, 2012). *P. lanceolata* is a common species in the study region and it was abundant in all strip treatments. Thus, it is possible that field margins also represent a source of RAA infestation counteracting potential positive effects of strip treatments mediated by higher natural enemy densities, notably at the orchard edge.

In both years a rapid and significant increase of aphid infestation was observed in April resulting in a high increment of aphid colonies and suggesting a relatively low natural regulation potential during early colonization. Such a strong and rapid increase of aphid abundance at the same period was also observed in an earlier study on RAA dynamics in orchards of the same region (Dib et al., 2010). An efficient biological control would require a sufficient number of natural enemies from April on. Most wildflower strip species started to flower later than April. In order to provide floral resources in time, a higher proportion of early flowering plant species is needed.

We found a positive relationship between aphid colony size and natural enemy density indicating that natural enemies within orchards mainly responded to the available amount of prey or hosts. However, natural enemy numbers did not significantly depend on aphid numbers in the wildflower strip treatment. In absence of aphids (regression intercept), the regression model predicted higher natural enemy densities for the wildflower strip treatment than for the other treatments which may be the result of a higher spill-over of natural enemies from the wildflower strip. A relatively high mobility of natural enemies attacking aphid colonies may compensate for a lower spill-over of natural enemies in the other strip treatments.

In conclusion, we found positive effects of wildflower strips on the biological control of aphids but the effects were less strong than in other studies using annual crops as model system (Pfiffner et al., 2009; Tschumi et al., 2015). However, most of these studies used less attractive controls to evaluate wildflower strip effects (absence of vegetation, crop strips) increasing the probability to obtain significantly positive results (Haaland et al., 2011; Tschumi et al., 2015). Additionally, spontaneous vegetation also occurred inside our orchards which may have attenuated differences between margin strip treatments. We are convinced that a successful conservation biological control approach needs to provide a significantly

better control service than spontaneously occurring vegetation to justify higher sowing and management costs. In order to improve regulation by wildflower strips, sowing inside orchards should be considered in order to reduce the distance of movements required to reach the target pest species. Our study also demonstrated the importance of temporal dynamics and the need to provide floral resources at the beginning of the season for an early control of the RAA colonies. Future studies on natural enemy and aphid movements and on their temporal dynamics are required to improve our mechanistic understanding of interactions between natural enemies and rosy apple aphids and to evaluate efficiency of wildflower planting orchard margins.

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CHAPTER IV- TRACKING THE MOVEMENT OF CODLING MOTH PREDATORS FROM WILDFLOWERS STRIP INTO APPLE ORCHARDS

RESUMO

O uso de plantas entomófilas é uma maneira de manter e conservar os inimigos naturais da praga em áreas adjacentes às culturas agrícolas e de aumentar o controle biológico natural. Neste trabalho, a hipótese de que faixas de flores adjacentes ao pomar de maçãs podem atrair predadores de solo e aumentar a sua dispersão dentro do pomar e o controle de *Cydia pomonella*. Nós pulverizamos proteínas de ovo (5%) nas faixas de flores adjacentes a dois pomares de maçã a cada 15 dias durante oito semanas para monitorar os movimentos predadores de solo. Selecionamos 6-9 macieiras em 6-9 linhas diferentes por pomar para monitorar conjuntamente a dinâmica dos predadores. Os artrópodes coletados em armadilhas pitfall foram levados para o laboratório e congelados para preservar seu DNA. Primeiro, eles foram identificados. Em segundo lugar, teste ELISA foi realizado para testar a presença de proteína de ovo. Em terceiro lugar, a presença de ADN de *C. pomonella* no estômago de cada predador foi testada utilizando um protocolo baseado em PCR. No total, 37 dos 490 indivíduos foram marcados pela proteína do ovo e 111 foram positivos à presença de DNA de *C. pomonella* no estômago. No entanto, apenas 4 indivíduos foram positivos à presença tanto de proteína de ovo quanto de DNA de *C. pomonella*. Concluímos que entre os artrópodes terrestres que estavam envolvidos na população de *C. pomonella*, poucos cruzaram as faixas de flores durante o experimento. Estudos utilizando técnicas de marcação são ainda necessários para compreender os movimentos de artrópodes entre as culturas e as infra-estruturas agroecológicas.

ABSTRACT

The use of entomophilous plants is a way to maintain and preserve natural enemies of pest insects in areas adjacent to crop fields and to enhance biological control. In this study we hypothesized that wildflower strips adjacent to apple orchards may attract ground predators into the orchard to control *Cydia pomonella* caterpillars. We sprayed ovalbumin (5%) every 15 days on the wildflower strips adjacent to two apple orchards for a period of eight weeks in order to analyse ground predator movement. We placed pitfall traps at different distances and captured ground beetles and spiders as major generalist predators. ELISA was performed to test the presence of ovalbumin on the captured predators. Third, the presence of *C. pomonella* DNA in the gut of each predator was estimated by genetic marker analysis. In total, 37 out of 490 individuals were marked by the egg protein and 111 were positive to the presence of *C. pomonella* DNA in their stomach. However, only 4 individuals were positive to the presence of both ovalbumin and *C. pomonella* DNA. We conclude that among the ground arthropods that were associated in the population of *C. pomonella*, only few crossed the flower strips during the experiment. Studies using marking techniques are still necessary to understand arthropod movements between crop and agro-ecological infrastructures.

RÉSUMÉ

L'utilisation de plantes entomophiles est un moyen de maintenir et de conserver les ennemis naturels des ravageurs dans les zones adjacentes aux cultures agricoles et d'améliorer le contrôle biologique naturel. Dans ce travail, nous avons émis l'hypothèse que les bandes de fleurs adjacentes au verger de pommiers peuvent attirer les prédateurs du sol et améliorer leur dispersion au sein du verger et le contrôle des chenilles *Cydia pomonella*. Nous pulvérisons des protéines d'œufs (5%) sur les bandes de fleurs adjacentes à deux vergers de pommes tous les 15 jours pendant huit semaines pour surveiller les mouvements des prédateurs au sol. Nous avons choisi 6-9 pommiers en 6-9 rangées différentes par verger pour surveiller conjointement la dynamique des prédateurs d'arthropodes. Les individus arthropodes recueillis dans des pièges ont été emmenés au laboratoire et congelés pour préserver leur ADN. Tout d'abord, ils ont été identifiés. Deuxièmement, ELISA a été réalisée pour tester la présence de protéines d'œufs. Troisièmement, la présence d'ADN de *C. pomonella* dans l'estomac de chaque prédateur a été testée en utilisant un protocole basé sur la PCR. Au total, 37 des 490 individus ont été marqués par la protéine d'œuf et 111 sur 490 étaient positifs à la présence d'ADN de *C. pomonella* dans leur estomac. Cependant, seuls 4 individus étaient positifs à la présence à la fois d'une protéine d'œuf et de l'ADN de *C. pomonella*. Nous concluons que parmi les arthropodes terrestres qui ont été impliqués dans la population de *C. pomonella*, seuls quelques-uns ont traversé les bandes de fleurs au cours de l'expérience. Des études utilisant des techniques de marquage sont encore nécessaires pour comprendre les mouvements d'arthropodes entre les cultures et les infrastructures agro-écologiques.

INTRODUCTION

Conservation biological control (CBC) involves semi-natural habitat management to enhance the survival, fecundity, longevity, and behavior of natural enemies in order to increase their efficiency in controlling pest insects. Provisioning of supplementary resources, manipulating host plant attributes or increasing landscape complexity are beneficial to natural enemies and may enhance pest control (Géneau et al. 2012).

The use of entomophilous plants within the concept of conservative biological control appears as a strategy to promote natural enemies and to reduce pesticide use (Griffiths et al. 2008). Field margins are important habitats of natural enemies attacking insect pests of adjacent crops since they provide shelter and complementary nutritional resources. However, their attractiveness may also prevent natural enemies from moving into the crop fields. In order to evaluate the contribution of margin vegetation to biological control, a better understanding of movements between margins and fields is required (Wanner et al., 2006). Arthropod movements in agroecosystems have been studied through different methods. Currently, the tracking of movements depends on methods to mark key arthropods (Jones et al. 2010; Hagler and Jones, 2010). Field experiments involving the labelling of target predators allow the identification of spatial dynamics and distribution patterns. Understanding predator movements may contribute to improve conservation biological control by managing the distribution of crop and semi-natural habitats in agro-ecosystems (Mönkkönen, 1999).

In this study, we apply a relatively new method to track predator movements using protein labelling of field margin vegetation (Jones et al. 2006). Proteins such as ovalbumin and casein are usually absent in such an environment and their detection in predator samples by an ELISA (Enzyme linked Immunosorbent Assay) test would confirm that these predators visited the field margin before capture. The method was successfully applied in studies on pest insect and natural enemies in fruit orchards (Horton et al. 2009, Basoalto et al. 2010,

Lefebvre, 2016). Additionally, we used a genetic marker analysis to test whether the captured and protein-labelled predators fed on codling moth as the most important pest insect in apple orchards. The combination of protein labelling and genetic marker analysis allows tracking predator movements from field margins into the field and testing whether those individuals predated codling moths.

The aim of this study was to track the movement of ground predators (spiders, carabids, and harvestmen) from wildflower strips into apple orchards, and to evaluate the contribution of these predators to the regulation of codling moth.

MATERIAL AND METHODS

Field sites, design and sampling

The experiment was set up in two experimental apple orchards of the INRA Saint-Paul field station, Montfavet, France (43°54'51.57"N, 4°52'56.15"E) and was run from August to September 2015. The first orchard comprised five rows of 24 apple trees, the second six rows of 48 apple trees. The margin of the two orchards had been sown with a wildflower strip mixture in the previous year. The mixture comprised 30 plant species of which 15 were present in 2015. No insecticides were applied during the study period.

Pitfall traps were placed at a distance of 20 cm from each apple tree trunk resulting in a total of 54-60 traps per orchard. After exposition overnight (12 hours) the traps were emptied. Arthropods were individually sampled to avoid protein contamination between samples (Jones and Unruh 2009). All arthropod individuals were kept in a freezer at -18°C in 1.5 mL microtubes until ELISA test. One week before the marker application in the wildflower strips, arthropods were collected in both the apple orchard and their margins. These arthropods were considered as controls.

Four liters of a 5% solution of egg protein (ovalbumin) were applied using a costal sprayer. The treatment was repeated every 14 days during eight weeks.

Indirect ELISA

Each arthropod individual was washed in 1 mL Phosphate Buffered Saline solution (PBS) and the presence of ovalbumin antigen was assessed using indirect ELISA (Jones et al. 2006). Commercial SIGMA© anti-bodies were used: the anti-chicken egg albumin produced in rabbit (C6534) was diluted to obtain anti-body concentration of 10^{-3} mg/mL. Each sample was tested twice on different plates. Optical densities (OD) were measured at 450 nm with a micro plaque reader (TECAN© infinite M200). Raw OD readings were corrected (correctOD) in each plate by removing the mean OD values of 16 wells with only PBS and without antigen. Arthropod individuals were considered as marked when their optical density was higher than the correct OD of negative controls plus four standard deviations in both ELISA plates (Jones et al., 2006, Sivakoff et al., 2011).

DNA marker analyses

To assess the codling moth consumption by carabids, spiders and opilions, single plex PCR was carried out with specific *C. pomonella* primers. All caught predators were tested. Total DNA of each predator individual was extracted using the DNeasy® 96 blood and tissue kit (Qiagen, Germany). The predators were not dissected before extraction. DNA extractions were performed on the entire body of each predator that was ground using an agitator-ball mill. PCR was performed separately on each predator using specific codling moth DNA primers. PCR mixtures were carried out in 10 μ L reaction volumes containing 2 μ L of DNA

template, one unit of Flexi GoTaq® (Promega), 2.5 mM MgCl₂, 0.1 mg/ml Bovine Serum Albumin, 20 mM (NH₄)₂SO₄, 200 mM of each dNTPs, 1X Promega PCR buffer, and 0.5 μM. PCR amplifications were performed on a Mastercycler thermocycler under the following conditions: initial denaturation at 95°C for 3 min followed by 35 cycles at 95°C for 30 s, 52°C for 30 s, and 72°C for 30 s and a final elongation step at 72°C for 20 min. Each PCR product was diluted (0.4 or 1 % dilution with 0.02% GeneScan™- 600 LIZ® Size standard in HiDi formamide, Applied Biosystems) and 2 μL of this dilution was injected on an ABI 3730xl DNA Analyzer. Capillary electrophoresis was performed using POP-7 polymer to detect amplifications of codling moth DNA, which were scored using the Genemapper® V4.1 software (Applied Biosystems). Amplification of codling moth DNA in the predator samples were compared with amplifications performed in the same PCR plate with 1 pg of codling moth DNA (positive control) and without DNA template (negative control).

These controls were used to verify the sensibility of each PCR run and the absence of DNA contamination during the biomolecular process. In order to reduce the assessment risk of false positive samples, a predator sample was considered positive to codling moth DNA when the intensity signal of its PCR product was above the mean intensity of the two controls.

RESULTS

A total of 490 arthropod individuals was collected during the study period. Among sampled ground predators 53.3% were beetles (91% of which carabids), 8.3% opiliones, and 38.2% spiders.

Codling moth DNA was found in the gut of 111 individuals (22.4%). Focusing on codling moth predators, we detected codling moth DNA in 57 individuals (33.1%) of the carabids. The most dominant carabid species was *Harpalus rufipes* (42 individuals), followed

by *Platysma vulgare* (13 individuals), and *Pterostichus niger* (two individuals). Furthermore, 35 spider individuals out of 187 (35 Lycosidae) and 13 opiliones individuals out of 41 were identified as predators of codling moth. In general, the DNA-positive codling moth predators were evenly distributed in the orchards (Fig.2).

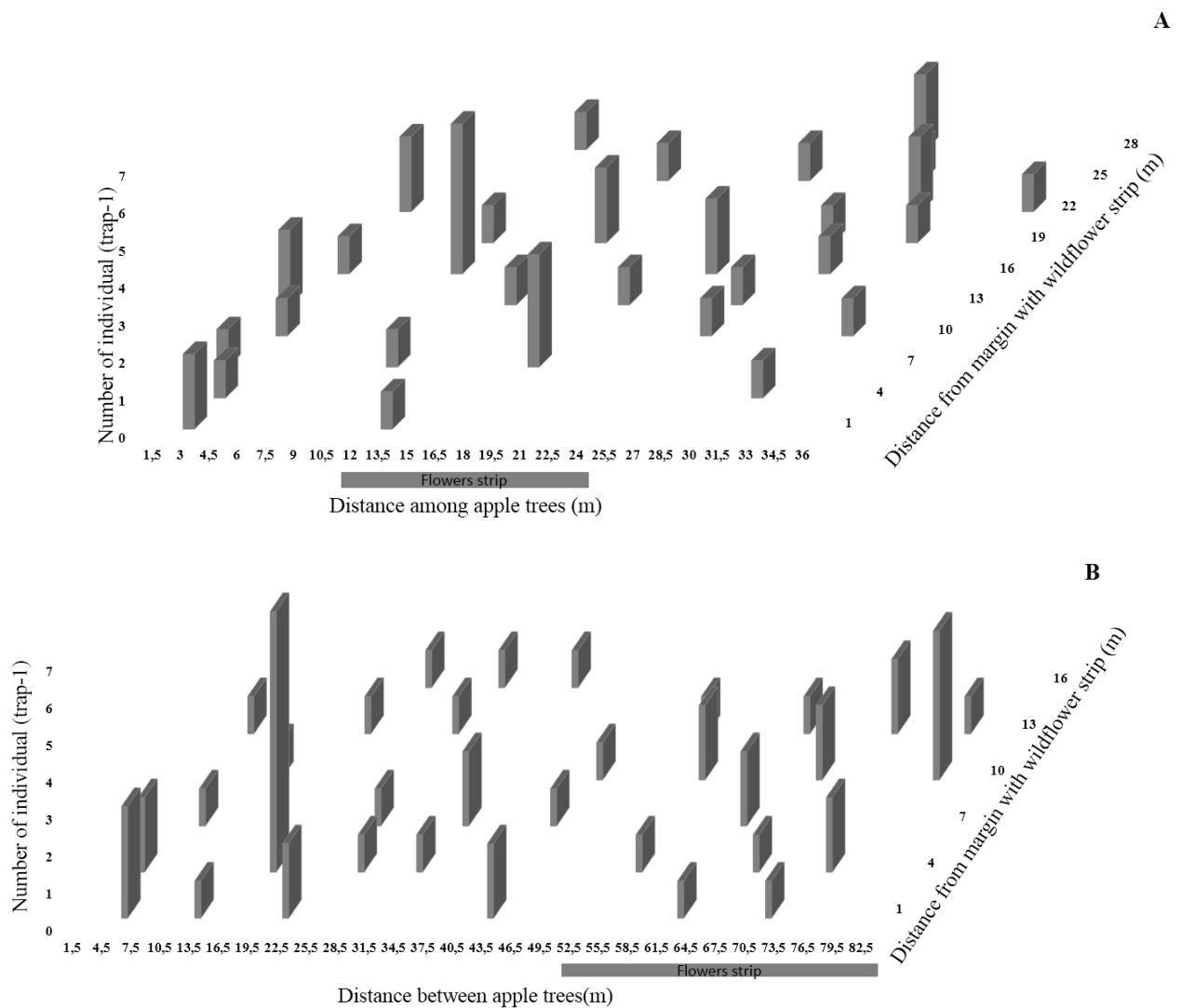


Figure 2. Spatial distribution (meters) of ground predators positively to codling moth DNA in orchards A (variety Ariane) and B (varieties Gala and Granny).

Among the arthropods captured in pitfalls traps, 37 arthropods (7.5% of total individual number) were marked with ovalbumin: 25 carabids (20 *Harpalus rufipes* (De Geer, 1774), 2 *Platysma vulgare* (Linne, 1758.), 2 *Pterostichus niger* (Schaller, 1783) , and one

unidentified specimen); seven spiders (Lycosidae) and five opilions. The ovalbumin-labelled predators were found evenly across all distances (Fig. 3). The number of labelled predators was not higher close to the labelled wildflower strips than at the opposite side of the orchards at distances between 45 and 55 m.

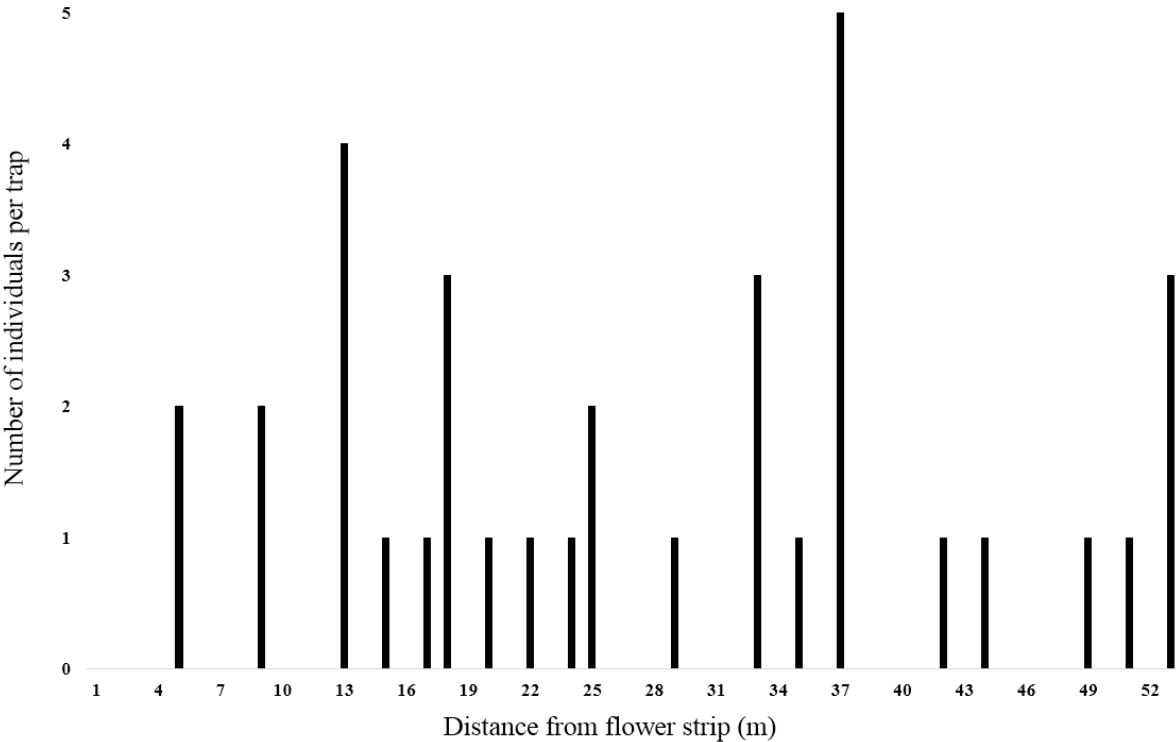


Figure 3. Number of ovalbumin marked predators at different distances from the wildflower strips.

Codling moth DNA was detected in only four ovalbumin-positive individuals (two *H. rufipes*, one Lycosidae, one unidentified). These individuals were found at distance of 8-37 m from the wildflower strip.

DISCUSSION

The main ground predators captured in pitfall traps were carabid beetles belonging to the genus *Harpalus*. Carabids may be generalist predators or specialised on prey groups herbivorous (Kromp, 1999). Field studies showed that carabid species feed on Tortricidae larvae when they are moving down to the ground for diapausing or pupation (Boreau de Roince et al., 2012). Mathews et al. (2004) studied the predation of codling moth sentinel larvae on the ground in newly planted apple orchard and found that predation rates varied between 34 % and 81% depending on the season and ground management.

In addition, spiders and opillions were marked suggesting that these soil predators use wildflower strips as habitats and move into the orchards. Spiders are important generalist predators, feeding on pests in the canopy of plants or on the ground (Brown et al., 2003). Markó et al. (2014) observed that wildflower strips may increase spider abundance in apple orchards. Information on oppilions and their role as predators in agrosystems are scarce.

We found a relatively low ratio of olbumin predators in apple orchards (7.5%). The result showed that few predators moved from the wildflower strip into the field margin.. However, the labelled individuals were found all over the tested orchards suggesting a high mobility of those predators coming from the field margins. Such a high mobility of ground beetles was also found in previous studies (Collins et al. 2002). The limited quantity of movements may be explained by a higher attractively of margin habitats preventing predators from moving into the orchards but also by competition with predators already occurring inside the orchards when field margins were labelled. Spontaneous vegetation occurring within the orchard may have provided sufficient resources and other habitat functions to maintain a high predator density (Lavandero et al. 2005) and Skirvin et al. (2011). Ground beetles, spiders and harvestmen do not directly depend on floral resources, and beetle banks suggested to increase ground beetle populations are based on grass mixtures (Collins et al. 2002). Thus wildflower

strips do not necessarily increase the abundance of these predators compared with spontaneous vegetation or grass strips (Luka et al., 2001).

We found ovalbumin-marked predators with *C. pomonella* DNA in the genetic marker study, even though in small amounts, but at far distances from the flower strip. Follow-up studies on the effects of these predators on regulation are necessary to evaluate the representativeness of the observed predator movements. Proving a sufficient movement of predators from wildflower strips into crop fields is indispensable to evaluate whether companion plants use is efficient in pest control (Ditner et al. 2013).

In conclusion, we conclude that among the ground arthropods that were associated in the population of *C. pomonella*, only few crossed the flower strips during the experiment. Studies using marking techniques are still necessary to understand arthropod movements between crop and agro-ecological infrastructures.

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CHAPTER V: FINAL CONSIDERATIONS

Habitat management is an important tool for natural biological pest control. For example, the maintenance and conservation of entomophilous plants allows natural enemies to occupy adjacent areas and to migrate into the crop for feeding or oviposition. Therefore, I studied the contribution of natural and semi-natural habitats for the attraction of natural enemies in two important crops: sugarcane (Brazil) and apple (France). In Brazil, the study comprised plateau and river valley forest fragments at two different dates after disturbance by sugarcane harvest. In France, the study compared wildflower strips optimized for pollen and nectar production, grass strips and strips of spontaneously occurring plants. In both countries, those habitats were located adjacent to crop fields.

In Brazil, preservation of 20% of farm's land must farmers kept to non-cropped (semi-) natural habitats to preserve biodiversity. However, farmers are usually unaware of ecosystem services provided by these habitats. Thus, in the chapter II, I studied the contribution of forest fragments as important non-crop habitats for functional diversity in sugarcane fields. A greater diversity and frequency of omnivorous and predatory ants close to the fragments were found. Also, diversity and frequency of ant species within sugarcane fields decreased with increasing distance from fragments. These ants are important predators of pest insects such as the sugarcane borer. In addition, I found that the diversity of ants decreased after disturbance by harvest activities, and that a part of the community disappeared from sugarcane fields resulting in a lower similarity between forest fragment and crop field communities. The increase in similarity several months after the harvest suggests a recolonization of sugarcane fields from these (semi-)natural habitats. These habitats provide resources and protection for ant species allowing survival during disturbance by cultivation or low resource availability during the dry season. During the vegetative stage of sugarcane growing season (= rainy

season), abundance and frequency of ant species increased particularly inside sugarcane fields, probably due to the increase of food availability.

The effect of different field margin strips (wildflower strips, grass strips and spontaneous vegetation) on the control rosy apple aphid *Dysaphis plantaginea* in apple orchards was studied (Chapter III). The results showed no main effect of strip treatments on pest regulation but a significant treatment x distance interaction demonstrated that treatment effects depended on distance to the margins. Natural enemies were favored by wildflower strips rich in floral resources. However, aphid number was generally higher close to the margin suggesting that higher aphid colonization from the margin may counteract a better control by natural enemies. Also, flowering of wildflower strip plants and therefore resource provisioning to natural enemies may have occurred too late in the season to efficiently control apple aphids.

The lack of wildflower strip effects on natural enemies within the orchards – although significant in the margins – raises the question whether natural enemies attracted by floral resources are moving into the orchard. Wildflower strips were also marked with ovalbumin to evaluate generalist natural enemy movement (ground beetles, spiders, harvestmen) from wildflower strips into the orchards. Analysis of the labelled predators was also performed to verify whether they fed on codling moth *C. pomonella* larvae (Chapter IV) using genetic marker analysis. I found a low percentage of ovalbumin labelled predators, but these labelled individuals were captured throughout the whole apple orchard suggesting a high mobility. DNA marker analyzes showed that approximately 25% of the predators fed on *C. pomonella* DNA. However, few individuals were ovalbumin labelled and showed codling moth DNA markers at the same time. Even with low marking percentage, I was able to demonstrate that generalist predators use these wildflower strips before moving into the orchard to feed on pest insects.

In general, our results demonstrated the importance of natural and (semi-)natural habitats in the maintenance of natural enemy populations adjacent to crop fields. This was shown for different climatic regions and for the abundance and diversity of predators. In addition, although labelling rate was not high, I could show that natural enemies move from field margin vegetation into orchards.