COMMUNAUTE FRANCAISE DE BELGIQUE
ACADEMIE UNIVERSITAIRE WALLONIE-EUROPE
UNIVERSITE DE LIEGE – GEMBLOUX AGRO-BIO TECH

Push-Pull strategy to control aphids in Belgium and China

Qingxuan XU

(Push-Pull strategy in Belgium)

(Push-Pull strategy in China)

Promoteur : Prof. Frédéric Francis

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Abstract:

Wheat (*Triticum aestivum* L.) is one of the most cultivated crops in temperate climates. As its pests are mainly controlled with insecticides which are harmful to the environment and human health, alternative practices such as intercropping have been studied for their potential to promote biological control. Fifty original research papers were obtained from a systematic search of the peer-reviewed literature. Results from a vote-counting analysis indicated that, in the majority of studies, pest abundance was significantly reduced in intercropping systems compared with pure stands. However, the occurrence of their natural enemies as well as predation and parasitism rates were not significantly increased. Nevertheless, other practices could be combined with intercropping to favour natural enemies and enhance pest control.

Dispersal of viruses is intimately tied to their vectors. Aphids are known to invest in costly antipredator behavior when perceiving cues of predators. Before presenting the results and the answers to the previous questions, a brief research was conducted in order to have an overview of the intercropping on the spread of aphids, to assess the potential impact of intercropping systems attracting natural enemies on the virus transmission. We studied aphid antipredator behavior in intercropping with wheat-broad bean (*Vicia faba* L.) as a model. The bird cherry-oat aphid, *Rhopalosiphum padi* Linnaeus, is an important vector of the barley yellow dwarf virus. The effects of two natural aphid enemies, adult and larvae of the seven-spot ladybeetle, *Coccinella septempunctata* Linneaus, on *R. padi* dispersion was studied under laboratory conditions. Results show that in receptor lines (other lines than the source one), two hours after the experiment started, aphids were more abundant in monoculture than intercropping in the presence of ladybeetle adults and larvae and after 24 hours, it was still the case in the presence of predatory larvae. These results might be explained by the non-host plant chemical cues and the physical barrier that was broad-bean plants confusing *R. padi* when searching for their host plants after being dropped from wheat by predators.

After make sure that the intercropping can reduce the dispersal of aphids in the presence of predators, *in fine* potentially limiting virus dispersal, especially shortly after aphids colonize plants. Then we try to solve how to increase the number of natural enemies of intercropping in Belgium and China.

Semiochemical substances have been tested to enhance biological control, with inconsistent results. Combining semiochemical and intercropping can be an interesting way to maximize pest control. In Belgium, a two-year setup involving wheat–pea strip intercropping combined with the release of *E*-β-farnesene (EBF) or methyl salicylate (MeSA) was tested as a push–pull

strategy to simultaneously repell aphids and attract beneficials. Two types of slow-release formulation (i.e., oil and alginate beads) containing EBF or MeSA were deployed with intercropping. The abundance of aphids was significantly decreased, hoverfly larvae and mummified aphids increased on both pea plants and wheat tillers by the release of oil-formulated EBF and MeSA. The proportion parasitism of aphids-parasitism rate was also increased by treating both crops in both years. Releasing EBF through oil rather than alginate beads proved significantly better for attracting natural enemies and reducing aphids. Aphids were negatively correlated with the density of hoverflies (both adults and larvae) and numbers of mummies. We also tested the combining in China and the experiments were set-up: wheat-pea strip intercropping solely, intercropping combined with the release of EBF, and intercropping combined with the release of MeSA, each treatment repeated three times. The total number of aphids throughout the growing season was significantly decreased in treatments with releases of semiochemicals compared to intercropping solely. The effect was stronger with MeSA than with EBF on the control of *R. padi*, and hoverflies and lacewings were twice more numerous in MeSA.

All the results showed that combining intercropping with the release of EBF or MeSA formulated in oil can significantly reduce aphid density and attract their natural enemies. Therefore, the combination of both strategies could help farmers reduce the use of insecticides.

摘要

小麦（*Triticum aestivum* L.）是温带气候中栽培的最重要的作物之一。小麦上的害虫主要是采用化学杀虫剂控制的，对环境安全和人类健康十分有害，因此，作物间作措施作为了一种有效的生物防治方法，近年来得到了广泛的研究。通过文献进行系统检索，筛选获得了 50 份与间作相关的研究论文，对论文的研究进行了分类统计以及结论分析。结果表明，与作物单作相比，作物间作可以减少害虫的发生数量，然而，综合分析结果表明间作对害虫天敌数量和寄生蜂的寄生率并没有显著增加。因而，有你要寻找另外一些生物防治方法与作物间作结合，以增加害虫天敌的数量，加强对害虫的控制作用。

病毒的传播与它们的载体密切相关。众所周知，蚜虫在感知到捕食天敌时，会相应的产生抗捕食行为如逃逸、坠落等。因此我们首先进行了一项简要的研究，观察在间作系统中蚜虫抗捕食行为的发生情况，以评估该行为在作物间作系统中对病毒传播过程的潜在影响。我们研究了蚜虫在小麦-蚕豆（*Vicia faba* L.）间作系统中的抗捕食行为。蚜虫选用的是大麦黄矮病毒的重要传播载体-禾谷缢管蚜。在实验室条件下，分别用七星瓢虫（*Coccinella septempunctata* L.）成虫和幼虫处理对禾谷缢管蚜（*Rhopalosiphum padi* L.）在作物间作系统中扩散的情况进行了研究。结果表明，释放瓢虫成虫或者幼虫 2 和 24 小时后，间作处理中扩散行上的蚜虫数量明显少于单作处理。我们认为间作系统中蚜虫由于抗捕食行为从寄主作物上坠落或者逃离后，由于非宿主植物的化学挥发物的干扰和物理障碍干扰了蚜虫继续就近选择合适的寄主植物，因此在扩散行上观察到的蚜虫的数量明显减少了。

当确定了间作系统中引入天敌不会因为蚜虫的抗捕食行为造成潜在的病毒扩散后，下一步我们将在比利时和中国农田中解决提高间作系统中天敌种群数量。

化学信息物释放是一种可以有效吸引天敌的物质，因此，将作物间作和化学信息物释放结合有可能进一步增强对害虫的生物防控作用。在比利时，我们将蚜虫报警信息素和水杨酸甲酯释放与小麦-豌豆间作处理联合起来，连续两年测试该“推-拉”系统对蚜虫及其天敌的影响。同时也试验了两种不同的缓释剂型：挥发油和微囊缓释球。实验结果表明，与单纯小麦-豌豆间作相比，化学信息物释放和间作联合可以进一步降低害虫种群数量，同时显著提高天敌种群的发生量和提高蚜虫寄生蜂的寄生率，此外结果还显示报警信息素挥发油比微囊缓释球缓释处理更有效。我们同时也在中国的河南进行了化学信息素和间作联合处理：小麦-豌豆间作、小麦-豌豆间作联合报警信息素挥

发油释放和小麦-豌豆间作联合水杨酸甲酯挥发油释放 3 个处理，田间调查结果显示，化学信息素和间作联合处理不仅可以显著的降低蚜虫的发生量，同时有助于天敌种群数量的提高，结果还显示水杨酸甲酯缓释处理比蚜虫报警信息素缓释处理更有效。综合两地的实验结果我们可以得出化学信息素和间作联合处理是一种更有效的生防措施，可以帮助农户减少化学农药的使用。

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Index

List of figures

List of tables

Chapter I General introduction

Wheat (*Triticum aestivum* L.) is important crops for the people of the world as well as Belgium and China. Aphids are among the most abundant and destructive insect pests of agriculture, particularly in temperate regions, their feeding can directly and indirectly damage the crop and influence yield, and they can vector virus (Rossing, 1991). Among aphid species, *Metopolophum dirhodum* (Walker), *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* (Linnaeus) attack a range of small grains (Black and Eastop, 2000), causing economic damage and necessitating routine insecticide use.

Pesticides have been widely applied to protect agricultural crops since the 1940s, and since then, their use has increased steadily. As more attention has been paid to sustainable agricultural production that reduce reliance on the pesticide use and associated economic, environmental, and health costs, more studies on integrated pest management focus on ecological function of volatiles released by plants and intercropping with leguminous crop on herbivores and their natural enemies in agroecosystems. Indeed, pesticides were largely reported to induce human diseases and to be harmful for the environment (Witzgall, 2001).

To reduce reliance on this pesticide use and associated economic, environmental, and healthy costs, we tried to promote the application of infochemicals and intercropping as efficient biological control agents by developing alternative strategies for aphid biological control in wheat field. Several methods can be considered to limit aphid populations on cereals, such as semiochemicals (Verheggen et al., 2008), intercropping (Lopes et al., 2015) as efficient biological control agents by developing alternative strategies for aphid biological control in wheat field.

Crop intercropping or mixing as a traditional agricultural technique for preventing crop yield decrease from plant disease and pests infestation in different world geographical areas. Intercropping, defined as a kind of multiple cropping system with two or more crops grown simultaneously in alternate rows in the same area. It was the agronomic practice for the development of sustainable food production systems (Agegnehu et al., 2006; Eskandari & Ghanbari, 2010), plays an important role in controlling pests and protecting beneficial insects relevant to enhancing biodiversity in an agroecosystem. Intercropping of cereals has a 1000-year old tradition in China and it is still widespread in modern Chinese agriculture.

The use of predatory insects, including coccinellids and chrysopids, as biological control agents to suppress pest populations on crops is widely accepted and recognized by the general public. Volatiles produced by hosts and plants, especially herbivore-induced plant volatiles (HIPVs), play an important role in the foraging behavior of parasitoids and may influence their search for hosts (Price et al. 1980). Semiochemicals that recruit predators and parasitoids (parasites that kill their hosts), or in other ways manage beneficial organisms, can be released by crop or companion plants, thereby providing new approaches to exploiting biological control of pests (Pickett et al., 2014). Some insect species, when attacked by natural enemies, release alarm pheromones, causing avoidance or dispersal behavior in conspecifics (Teerling et al., 1993, Macdonald ert al., 2002). The alarm pheromone for many pest aphids is (E)-β-farnesene . It can be applied to the main crop to repel aphids in the field (Zhou et al., 2016).

Recently it has been observed that use of intercropping and semiochemical, may hold potential to manipulate an agroecosystem in a push-pull or stimulodeterrent diversionary strategy. Push-pull strategies involve the behavioral manipulation of insect pests and their natural enemies via the integration of stimuli that act to make the protected resource unattractive or unsuitable to the pests (push) while luring them toward an attractive source (pull) from where the pests are subsequently removed.

The principles of the push-pull strategyare to maximize control efficacy, efficiency, sustainability, and output, while minimizing negative environmental effects. Each individual component of the strategy is usually not as effective as a broad-spectrum insecticide at reducing pest numbers. Intercropping is one way to increase plant diversity to control aphids (Poggio 2005). In addition to intercropping, semiochemicals are particularly interesting in push-pull strategies (Miller & Cowles, 1990). A large guild of parasitoids and predators in agro-ecosystems are increasingly recognized as important sources of biocontrol for invasive agricultural aphids. Whereas intercropping alone may not enhance pest natural enemies, the use of semiochemicals in monocultures may not be consistently successful and may even negatively influence natural enemies in low pest density situations (Wang et al., 2011). Combining semiochemicals with intercropping may bridge these problems. In the context of aphid natural enemy attraction, wheat-pea strip intercropping combined with the release of E-β-farnesene or methyl salicylate was tested as a push-pull strategy to simultaneously repell aphids and attract beneficials.

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Chapter II Wheat (*Triticum aestivum* L.)-based intercropping systems for biological pest control- A review

Wheat (*Triticum aestivum* L.)-based intercropping systems for biological pest control

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Abstract

Wheat (*Triticum aestivum* L.) is one of the most cultivated crops in temperate climates. As its pests are mainly controlled with insecticides which are harmful to the environment and human health, alternative practices such as intercropping have been studied for their potential to promote biological control. Based on the published literature, this study aimed to review the effect of wheat-based intercropping systems on insect pests and their natural enemies.

Fifty original research papers were obtained from a systematic search of the peer-reviewed literature. Results from a vote-counting analysis indicated that, in the majority of studies, pest abundance was significantly reduced in intercropping systems compared with pure stands. However, the occurrence of their natural enemies as well as predation and parasitism rates were not significantly increased. The country where the studies took place, the type of intercropping, and the crop that was studied in the association had significant effects on these results.

These findings show that intercropping is a viable practice to decrease insecticide use in wheat production systems. Nevertheless, other practices could be combined with intercropping to favour natural enemies and enhance pest control.

Key words: sustainable agriculture, crop diversity, conservation biological control, predators, parasitoids, yield

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops worldwide (ranked fifth in terms of production according to FAOSTAT (http://faostat3.fao.org/browse/Q/QC/E)). Therefore, finding alternative methods to improve its sustainable production is a major challenge for today's agriculture. Conventional farming practices contributed to increase yields during the 20th century, but are today contested for their negative impact on the environment (Gibbons et al., 2015; Krebs et al., 1999) and human health (Baldi et al., 2013). Industrialized monoculture systems, which are highly dependent on the use of external inputs such as agrochemicals (i.e. synthetized fertilizers, chemical pesticides, growth regulators), favoured the simplification of agroecosystems (Kremen et al., 2012; Malézieux, 2012).

In contrast, promoting functional biodiversity, which supports ecological processes, may allow agricultural systems to benefit from various ecosystem services, including nutrient cycling, soil structuration and pest control (Altieri and Rosset, 1996; Zhang et al., 2007b). One of the 'agrobiodiversity strategies' to improve the sustainability of wheat production (reviewed by Costanzo and Bàrberi 2014) is to increase plant species diversity at the field scale though intercropping designs (Hauggaard-Nielsen et al., 2001; Malézieux et al., 2009a; Poggio, 2005a). Intercropping is defined as the cultivation of at least two plant species simultaneously in the same field (Andrews and Kassam, 1976; Anil et al., 1998; Ofori and Stern, 1987), but which are not necessarily sown and/or harvested at the same time (Lithourgidis et al., 2011b).

Andrews and Kassam (1976) categorised intercropping into four principle types based on the spatial and temporal overlap of plant species: (1) mixed intercropping - two or more crops mixed with no distinct row arrangement; (2) row intercropping - two or more crops grown in separate alternate rows (when plant species are alternated within the same row it is considered as within-row intercropping); (3) strip intercropping - several rows of a crop (strip) alternated with several rows of one or more other crops; (4) relay intercropping - two or more crops grown in relay, but with the growth cycles overlapping to some degree. Choosing a type of intercropping may depend on the associated crops and their valuation after harvest, in addition to the knowledge of the famer and the level of mechanisation used.

Intercropping systems tend to produce higher yields compared to monocultures and reduce the impact of agriculture on the environment. Specifically, intercropping may improve soil conservation, fertility and crop quality, while possibly reducing the incidence of weeds, disease and insect pests (Aziz et al., 2015; Bedoussac et al., 2015a; Lithourgidis et al., 2011b). Focusing on pests, as stated in the 'resource concentration hypothesis' from Root (1973) specialist herbivores are more likely to find their host plants when they are concentrated in dense or pure stands. Moreover, according to the ‘enemy hypothesis’ from Root (1973) the suppression of herbivores by their natural enemies (i.e. predators and parasitoids) is expected to be more efficient in diversified crop habitats compared to simplified ones, as they may be more abundant in environments offering a greater diversity of prey/host species and microhabitats to exploit.

Although the effect of intercropping on pests and natural enemies have been largely covered in the literature (Andow, 1991; Dassou and Tixier, 2016; Langellotto and Denno, 2004; Letourneau et al., 2011b; Risch, 1983; Tonhasca and Byrne, 1994), most comprehensive reviews are very generalists. As wheat is one of the most important crops worldwide, understanding the potential of wheat-based intercropping systems for biological control may be of crucial importance. More specifically, this study aimed at answering the following questions: (i) Are pests reduced and natural enemies favoured in wheat-based intercropping systems compared to pure stands? (ii) Is there a correlation between biological control and yield in wheat-based intercropping systems? (iii) Where and when were these systems studied? (iv) What are the technical characteristics of wheat-based intercropping systems (i.e. types of intercropping and plant species associated with wheat)? Overall, this study is expected to give valuable information about the potential of intercropping as a tool to reduce insecticide use in wheat production.

2. Experimental methods

2.1 A systematic research of the literature

To locate scientific literature related to the effect of wheat-based intercropping on pests and/or natural enemies, all terms potentially related to intercropping, wheat, pests and natural enemies were listed. These terms were then included in a single query, as follows: (intercrop* OR "crop association" OR "crop combination" OR "combined crop" OR "associated crop" OR "crop mix" OR "mixed crop" OR "mixed cropping" OR “row cropping” OR “relay cropping” OR “strip cropping”) AND (wheat OR "triticum aestivum") AND (pest* OR herbivor* OR "natural enemy" OR predator* OR parasit*). The composed terms were put between quotation marks so that the entire term was considered. For some of them, an asterisk was used to include all words that have a common core. The first step of this research was completed on 26 June 2015 by introducing the query in the search engine from the University of Liège (ULg - Belgium) e-bouquet. The search engine includes several e-journals and databases such as Scopus (Elsevier), AGRIS, CAB Abstracts and ProQuest (for the list of all databases included, see Annex 1).

Thereafter, the search query was adapted to each database, as some of them use a specific query language.

The obtained references were then selected based on the abstracts of the published papers. The abstracts had to meet four criteria to be retained for further analysis. First, they had to be research papers from peer-reviewed journals. Review and meta-analysis papers were not considered, as they are based on other studies. Second, the abstracts had to focus on intercropping. As stated in the Introduction, intercropping was defined as the cultivation of at least two plant species simultaneously in the same field, without necessarily being sown and/or harvested at the same time. Wheat had to be included in the intercropping and associated plant species had to be harvestable and consumable (human consumption, animal feeding, energy production and fibres). Ornamental, grassy or woody species were excluded. Third, insect pests and/or natural enemies (i.e. predators and parasitoids) had to be assessed by the studies and the effect of biological control had to be specified through direct (e.g. predation or parasitism rate) or indirect (e.g. abundance) indicators. Finally, the intercropping had to be compared to a pure stand control treatment. When the abstract was not available, the paper was excluded from the review. When the information contained in the abstract was not sufficiently precise to respond to criteria, the full paper was analysed. The paper was excluded from analyses if it was not obtainable.

Selected papers were then analysed in greater depth to determine the country where the study took place, the plant species associated with wheat, the type of intercropping and the effect of intercropping on yield, insect pests and/or natural enemies. Concerning insects, the effect was considered to be negative, positive or neutral when their populations declined, increased or no significant difference was detected, respectively between treatments. Furthermore, an increase in the predation or parasitism rate was considered to be a positive effect on natural enemies. In fact, both indicators allow determining the top-down impact of predators or parasitoids on their herbivorous prey or hosts. Therefore, we considered that higher predation or parasitism rates mean higher pressure on pests, which is positive for biological control. In the event that a single paper showed positive, negative and neutral effects on different insect populations, crops and intercropping designs (i.e. strip, relay, mixed), all instances were considered, hereafter termed ‘responses’.

2.2 Vote-counting method

The analysis of the selected papers was performed following the vote-counting method, which considers the number of tests supporting a theoretical relationship (i.e. in our case, if pests are reduced and natural enemies favoured in intercropping systems, compared with pure stands).

Despite a wide use of this method for analysing results of numerous different studies (Connell, 1983; Denno et al., 1995; Garratt et al., 2011; Haaland et al., 2011; Root et al., 2003), vote-counting has been criticized and meta-analysis promoted (Letourneau et al., 2011b; Tonhasca and Byrne, 1994). Indeed, vote-counting presents some limits that were reviewed by Combs et al. (2011) However, vote-counting allows the analysis of a large amount of papers for which the precise data are not always available. It is the case for several papers retrieved from the literature search, which still provide valuable findings that are worth to be considered.

2.3 Statistical analyses

In order to perform statistical analyses, a score was given to each response. The score '1' was given when a positive effect on biological control was recorded (i.e. lower abundance of pests, higher abundance of predators or parasitoids, higher parasitism or predation rates, higher yield). The score '0' was given when no effect or a negative effect was recorded. The Exact Bernouilli test ($P < 0.05$) was used to assess whether the frequency of responses where intercropping had a positive effect on biological control compared to pure stands differed from that expected by chance. Generalized linear models (GLMs) with binomial error (logit-link function) were fitted to assess whether (i) the country where the study took place, (ii) the type of intercropping, and (iii) the crop species that was studied had effects on the responses. These variables as well as every possible interaction were tested using a likelihood-ratio test ($P < 0.05$). Finally, the Pearson correlation between the effect of intercropping on pests, natural enemies and yield was tested ($P < 0.05$). The analyses were performed using R software (R Core Team, 2013a).

3. Results

3.1 Countries and evolution through time

Out of 445 papers that were examined, 50 papers met the stated criteria. Thirty-nine of these papers were found using the search engine of the ULg. Eleven additional papers meeting the criteria were found by adapting the query to each database.

Four regions of the world are represented by the 50 studies. Twenty three were carried in China, 12 in Central and Southern Asia (i.e. India, Pakistan, Bangladesh and Iran), and 11 in North America (i.e. United States of America, Canada). Four papers refer to experiments carried in Western Europe (i.e. France, Denmark, Belgium and Germany) (Fig. 1). The oldest paper found was published in 1987 in China (Fig. 2). Since this year, one to two papers were published every year on average throughout the world. However, the number of publications increased from 2009 with 40 % of them published since this date. The first paper published in Europe was in Germany in 2006.

3.2 Plants associated with wheat and types of intercropping

Thirteen plant species were recorded in association with wheat (Table 1). The main species included cotton (*Gossypium* sp.), oilseed rape (*Brassica napus* L.) and pea (*Pisum sativum* L.). Different kinds of intercropping with wheat were implemented depending on the species used (Table 1). Strip cropping is the most common type, representing almost half of the studies, followed by relay cropping. Relay cropping was used when cotton, field bean (*Phaseolus vulgaris* L.), sorghum (*Sorghum bicolor* L.) or soybean (*Glycine max* L. Merr.) were associated with wheat. Mixed cropping was the least reported type. Pea, oilseed rape and faba bean (*Vicia faba* L.) were found mixed with wheat in this system.

3.3 Pests and their natural enemies

Forty-nine (98 %), twenty-four (48 %) and fourteen (28 %) papers assessed the effect of intercropping systems, compared to pure stands, on pests, predators and parasitoids respectively. Among them, twelve (24 %) considered both predators and parasitoids. Wheat-based intercropping systems significantly decreased pest populations compared to pure stands ($P < 0.001$), while no significant effects were observed for predators ($P = 0.480$) and parasitoids ($P = 0.359$) (Fig. 3).

Responses from pests and natural enemies varied significantly between countries where studies took place (Table 2). All responses obtained for pests in Bangladesh, Belgium, Denmark, France and Iran reported a decrease of their populations, while the opposite was observed in the only study that was carried in Germany. Variable responses were obtained in other countries, especially in the three Canadian studies (Fig. 1). As for natural enemies, the study that was carried in Iran was the only that reported an increase of predator populations or predation rate, while the opposite was observed in Belgium. As for pests, variable responses were observed in other countries. Similar results were obtained for parasitoids. The study from Pakistan was the only one reporting an increase of parasitoids abundance or parasitism rate, while a decrease was obtained in the single study from Canada.

Both pests and natural enemy responses were significantly affected by the type of intercropping (Table 2). Pest populations were always reduced in strip cropping, which also favoured predators and parasitoids more often than relay and mixed cropping. The latter reduced pests in half of the cases and never induced an increase of natural enemy populations, as well as predation and parasitism rates (Fig. 4). Finally, such variability of responses was also observed for pests and parasitoids, but not for predators, when considering the crop species that was studied in the wheat-based intercropping system (Table 2; see Table 3 for details and associated references). Pests were reduced on the majority of crops, but rarely on oilseed rape (Fig. 5).

Variable responses were obtained for other crops, especially sorghum, sugarcane (*Saccharum officinarum* L.) and mustard (*Sinapis albla* L.). Predators were not favoured on alfalfa (*Medicago sativa* L.), pea and sorghum, and a beneficial effect was recorded on cotton and wheat in only half of the cases (Fig. 5). The only study where oilseed rape was considered reported two opposite effects. As for parasitoids, all responses obtained with oilseed rape corresponded to a decrease of populations or parasitism rates, while more than a half of them were beneficial for biological control on cotton and wheat (Fig. 5).

3.4 Crop yield

The effect of intercropping on yield was assessed in only 10 of the 50 papers. Six papers reported significant higher yield in intercropping systems compared to pure stands, while a single one showed the opposite. Two of them reported no significant differences. Additionally, one paper reported significant higher yield in intercropping compared to pure stand in the first year and no significant differences in the following one. No significant correlation was found between pest reduction and yield increase ($\varphi = 0.45$, $P = 0.145$). However, higher yield was positively correlated with an increase of predator populations and predation rate ($\varphi = 0.77$, $P = 0.024$). This positive correlation was even stronger when predator and parasitoid data were analysed together ($\varphi = 0.81$, $P = 0.002$). However, not enough data were available to test such a correlation for parasitoids alone.

4. Discussion

4.1 Effect on pest biological control and implication for yield

4.1.1 Insect pests and natural enemies

Wheat-based intercropping systems almost systematically have a positive effect on pest control. In fact, the number of responses reporting a decrease of their populations was significantly higher than those showing the opposite. This finding is consistent with most studies addressing the effect of plant diversity on herbivores (Andow, 1991; Letourneau et al., 2011b). Most of the mechanisms explaining how plant diversity promotes pest regulation, called associational resistance (Tahvanainen and Root, 1972), were compiled by Poveda et al.(2008) and Barbosa et al. (2009). For example, pest ability to locate host plant odours may be disrupted when they are masked by volatiles from non-host plants (Tahvanainen and Root, 1972). Moreover, host plant odours may be altered when exposed to volatiles from neighbouring insect-infested (Ton et al., 2007) and non-infested (Ninkovic et al., 2013) plants, but also after absorbing certain root exudates from adjacent non-host plants (Finch and Collier, 2000). In some cases, competition between associated plants may alter the quality of host plants, which become less attractive for pests (Theunissen, 1994). Pests may also be more attracted to associated non-host plant species and remain on these plants without infesting the main crop (Vandermeer, 1989). Alternatively, certain plants have repellent odours (Uvah and Coaker, 1984). Other mechanisms may also affect the visual location of host plants, such as greener and/or taller non-host plants, which may camouflage the host plant (Finch and Collier, 2000) or even lead to its physical obstruction (Perrin and Phillips, 1978).

Furthermore, natural enemies may exercise a top-down control on pests. However, the number of responses reporting a beneficial effect of intercropping on predators and parasitoids was not significantly higher than the one reporting the opposite. This result is not consistent with the 'enemy hypothesis' of Root. Several explanations have been put forward by the authors of the analysed papers to explain that. For instance, according to Hummel et al.(2012) who found that canola-wheat intercropping did not increase ground beetle (Coleoptera: Carabidae) populations compared to pure stands, intercropping may have altered microhabitat conditions (i.e. soil moisture, temperature and light penetration through the canopy), making the environment less suitable for some species. The same authors also found that the parasitism rates of the root maggot *Delia radicum* (L.) puparia decreased with increasing proportions of wheat in a canola-wheat intercropping system. Since *Delia* spp. caused less damage in intercropping systems compared to pure stands, it was hypothesised that the amount of volatiles emitted by infested canola plants, which attract the adult parasitoid *Aleochara bilineata* Gyll., were limited by intercropping. A similar hypothesis was proposed by Lopes et al. (2015) to explain why adult ladybeetles and hoverflies were significantly more attracted by pure stands of pea and wheat, respectively, which were significantly more infested by aphids compared to mixed and strip cropping systems. Moreover, some practical aspects may explain that natural enemies were rarely favoured in intercropping systems. In relay-intercropping for instance, whereas this system may allow natural enemies to maintain though time, a lack of temporal overlap between the several crops may cause a dissipation of the natural enemies (Parajulee and Slosser, 1999). Also, the use of insecticides in experiments could have negatively affected natural enemies resulting in no differences between treatments (Chen et al., 1994). Landis et al. (2000) reported that plant diversity should benefit natural enemies partly because it may provide pollen and nectar that are alternative non-host food sources. However, a particular attention must be paid on the crop phenological and physiological characteristics that may affect natural enemies. Despite several flowering crops may produce such food sources (e.g. oilseed rape, alfalfa or faba bean with extra floral nectar), the flower architecture must be adapted to insect mouth parts (Campbell et al., 2012) and the resources must be available when they are needed (Colley and

Luna, 2000). These aspects may explain why simply associating crops do not necessarily favour natural enemies.

4.1.2 Crop yield

There was no significant correlation between pest reduction and yield increase. This result is consistent with Letourneau et al. (2011) who also found that beneficial effects of plant diversity on pest reduction are not systematically translated in higher yield. One reason is that the type of intercropping also influences other agronomic aspects, such as plant density and competition for resources. Yield may particularly be affected in substitutive designs like mixed intercropping, as they imply lower crop densities when compared to pure stands, but also higher competition for water, light and nutriments between associated plants (Letourneau et al., 2011b). However, according to Bedoussac et al. (2015), yield of all associated crops considered together is almost systematically higher compared to the one of each crop grown in pure stands. In our study, not enough data were obtained to fully address this question. However, we might hypothesize that minimizing the competition between intercropped plants can be achieved in relay and strip intercropping, which are also the most efficient for controlling pests and favouring natural enemies. The positive correlation between the beneficial effect of intercropping on natural enemies and higher yield may encourage following this direction. Furthermore, as noted by Letourneau et al.(2011), it would be interesting to determine whether eventual yield losses due to intercropping are compensated by environmental benefits and input cost reduction (in our case insecticides) in future studies.

4.2 Adopting intercropping for pest control: constraints and opportunities

4.2.1 A well-established practice in Asia that is beginning to take hold in Europe

Most studies addressing the effect of wheat-based intercropping on pests and/or natural enemies were carried out in China. Despite the fact that intercropping has been practiced in Chinese agriculture for over 1000 years (Knörzer et al., 2009a), there has been a strong decline in the use of this method on the North China Plain over the last 20 years (Feike et al., 2012). In fact, with the decrease of rural labourers and increase in farmer’s income, farmers have invested in mechanisation, adopting intensive production methods. As noted by Feike et al. (2012), one of the ways to overcome this issue is to replace the traditional labour-intensive row intercropping system by strip intercropping, which can be more easily adapted to mechanisation. Therefore, it is not surprising that many studies carried out in China have focused on this type of intercropping.

In contrast, studies remain rare on intercropping as a tool to biologically control pests in Europe. This may be because this practice needs technical adaptations (see section 4.2.2) to be implemented, which are not compatible with the conventional agriculture model that has been practiced in Europe for the last 30 years (Malézieux, 2012). In fact, for farmers, developing intercropping systems requires new skills and tools (Malézieux et al., 2009a). In addition, these systems must satisfy the ecological, economic and social constraints on their farms (Malézieux, 2012). However, the growing focus on low-input farming practices in academic environments (Doré et al., 2011; Malézieux, 2012; Wezel et al., 2014) and at the political level (De Schutter, 2010; Guillou et al., 2013) may explain the recent development of research on intercropping in Europe.

4.2.2 Adopting intercropping needs technical adaptations

Management and technical issues are central for developing intercropping systems. Indeed, phenological and spatial constraints of crop species must be taken into account to select viable combinations. Competition for resources (i.e. light, water, nutrient) (Thorsted et al., 2006), as well as allelopathic effects (Khan et al., 2002), may limit whether associations work. Appropriate machines are also needed to sow, harvest and separate grains in mixed cropping (Lithourgidis et al., 2011b). However, the management of strip and relay intercropping systems may be facilitated, as two or more crops may be separately managed. Also, the size of the strips and the ratio between the associated crops can be adapted depending on farmer production objectives and agronomic constraints (i.e. in the selected studies, the width of the strips went from few crop rows to at least 5 m. and the ratio between crops was from 1 to 4). This may explain why the majority of studies focus on these two systems. Among the crops associated in relay, the combination of wheat with cotton is widely practiced in China (Zhang et al., 2007a). As well described by Zhang et al. (2008), “the cotton is sown in April, approximately seven weeks before the harvest date of wheat. Strips are left open in the wheat crop at sowing (October/November) to provide space for the cotton plants during their seedling stage (April, May and June). After the wheat harvest in June, cotton plants can exploit the full space, above-ground as well as below-ground.” As for mixed intercropping, wheat was only found associated with pea and oilseed rape. Wheat-pea mixtures are known to provide many benefits. For instance, wheat benefits from the symbiotic nitrogen fixation of peas, allowing to reduce fertilizer inputs (Ghaley et al., 2005; Pelzer et al., 2012). Some experiments have been published on the effects of wheat-pea mixtures, but not necessarily on the aspect of pest control (Ghaley et al., 2005; Lithourgidis et al., 2011c; Pelzer et al., 2012). In comparison, studies on the effects of mixing wheat and oilseed rape seemed to be a rarer combination, at least based on the publication record.

4.2.3 Combining crops of primary importance to favour the adoption of intercropping

Intercropping systems involve cultivating two or more crops in the same place at the same time. However, one crop is often seen as more important than the other crops for economic reasons (Lithourgidis et al., 2011b). This issue may explain why intercropping was studied to mitigate pests and favour natural enemies for just one of the associated crops in most studies. Cotton, sugarcane and soybean are well-known important cash crops that are exported worldwide (FAOSTAT (http://faostat.fao.org/site/342/default.aspx)). A particular crop may also be of special economic and cultural importance in some regions, such as chili pepper (*Capsicum frutescens* L.) in China (Lu et al., 2011) or the oilseed rape variety Canola in Canada (Raymer, 2002).

Wheat is an essential food crop in northern China and central Asia (Carter and Zhong, 1999; Morgounov et al., 2007), as it is in Europe and North America (FAOSTAT (http://faostat3.fao.org/browse/Q/*/E)). However, wheat is rarely considered as the main crop in intercropping systems in Europe and North America. Because conventional farming practices applied to wheat production already tend to achieve high yields, producing wheat under intercropping systems may not be seen as needed for economic and food security reasons. However, it is necessary for agriculture to shift toward more ecological food production in Western countries. Developing intercropping systems that are beneficial for crops of primary importance may favour such a transition.

4.3 Needs for further research

This study shows that wheat-based intercropping systems allow reducing pest occurrence on crops, while natural enemies are not favoured in such systems when compared to pure stands. However these results varied significantly depending on the countries where the study took place, the type of intercropping and the crops studied. In Europe, more research is needed to better assess the potential of wheat-based intercropping for pest control. Despite some limiting factors, mixed intercropping deserves to be further studied, as it may also provide some benefits. Because predators and parasitoids are not significantly favoured in intercropping systems, these latter could be combined with other practices known to efficiently support natural enemies within fields. For instance, some volatiles known to attract natural enemies can be released in fields. Wang et al. (2011) showed that the abundance of ladybeetles and parasitism rate were higher when methyl salicylate was released in wheat-oilseed rape intercropping fields, compared to each treatment applied separately. Moreover, infrastructures such as woodlots, hedgerows and wildflower strips could be settled in farming areas as they are known to provide habitats sustaining natural enemies that prey on and parasitize pests in adjacent fields (Colignon et al., 2002; Haaland et al., 2011; Morandin et al., 2014). Among other factors, the regulation of pests by natural enemies depends on their presence in the surrounding landscape (Fahrig et al., 2015). The conservation of natural enemies and their attraction in intercropping fields could be a way to improve the biological control of pests.

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Figures and Tables:

Table 1. **Plant species associated to wheat based on the type of intercropping**

Type of intercropping	Crops associated with wheat	No. of papers	References
Strip cropping	Alfalfa (*Medicago sativa* L.)	4	(Fathi et al., 2013; Ma et al., 2007a; Saeed et al., 2013; Skelton and Barrett, 2005)
	Garlic (*Allium sativum* L.)	2	(Wang et al., 2008; Zhou et al., 2013a)
	Mung bean (*Vigna radiata* (L.) Wilczek)	2	(Xie et al., 2012b, 2012c)
	Oilseed rape (*Brassica napus* L.)	7	(Dong et al., 2012; Hummel et al., 2012; Sarwar, 2011; Sherawat et al., 2012; Wang et al., 2011a, 2008, 2009c)
	Pea (*Pisum sativum* L.)	4	Ehsan-Ul-Haq and Van Emden 2003; Zhou et al. 2009a, 2009b, 2013Ehsan-Ul-Haq and Van Emden 2003; Zhou et al. 2009a, 2009b, 2013(Ehsan-Ul-Haq and Van Emden, 2003; Zhou et al., 2009c, 2009d, 2013b)
	Chili pepper (*Capsicum frutescens* L.)	1	(Chen et al., 1995)
Relay cropping	Cotton (*Gossypium* sp.)	10	(Chen et al., 1994, 1998, Ma et al., 2006b, 2007b; Mu et al., 1993; Parajulee and Slosser, 1999; Parajulee et al., 1997; Wang and Zhao, 1993; Wang et al., 2009a; Zhao et al., 1987)
	Field bean (*Phaseolus vulgaris* L.)	1	(Tingey and Lamont, 1988)
	Sorghum (*Sorghum bicolor* L.)	1	(Phoofolo et al., 2010)
	Soybean (*Glycine max* (L.) Merr.)	2	(Hammond and Jeffers, 1990; Miklasiewicz and Hammond, 2001)
Mixed cropping	Oilseed rape (*Brassica napus* L.)	4	(Hummel et al., 2009b, 2009a, 2010; Paulsen et al., 2006)
	Bean (*Vicia faba* L.)	1	Hansen et al. 2008Hansen et al. 2008(Hansen et al., 2008)
Strip and mixed cropping	Pea (*Pisum sativum* L.)	2	(Lopes et al., 2015a; Ndzana et al., 2014a)
Non specified	Chickpea (*Cicer arietinum* L.)	3	(Das, 1998; Hossain, 2003; Mehto et al., 1988)

Cotton (*Gossypium* sp.)	2	(Xia et al., 2000; Zhang, 1990)
Bean (*Vicia faba* L.)	1	(Yang et al., 2009b)
Mustard (*Sinapis alba* L.)	3	(Ansari et al., 2007; Mishra et al., 2001; Tiwari et al., 2005)
Sugarcane (*Saccharum officinarum* L.)	1	(Masih et al., 1988)

Table 2. Effect of wheat-based intercropping on pests and natural enemies according to the countries where the studies took place, the type of intercropping and the crop of primary interest. Likelihood-ratio tests on GLMs; *P < 0.05; **P < 0.01; ***P < 0.001. '-' indicates that it was not possible to perform the analysis.

	Pests			**Predators**			**Parasitoids**		
Predictor variables	**df**	**χ^2**	**Pr(>Chi)**	**df**	**χ^2**	**Pr(>Chi)**	**df**	**χ^2**	**Pr(>Chi)**
Country	10	19.47	0.035 *	5	21.47	< 0.001 ***	2	7.61	0.0223 *
Type of intercropping[a]	2	18.39	< 0.001 ***	2	6.20	0.045 *	2	7.85	0.020 *
Crop	11	27.63	0.004 **	5	8.46	0.133	2	7.85	0.020 *
Crop*Type of intercropping[a]	–	–	–	–	–	–	–	–	–
Crop*Country	–	–	–	1	1.29	0.255	–	–	–
Country*Type of intercropping[a]	–	–	–	1	2.15	0.142	–	–	–

[a] papers where the intercropping design was not defined were not considered in the analysis

Table 3 Effect on pests, predators and parasitoids according to the plant species that was studied in the intercropping

Crop	Effect			No. of papers	References
	(-)	(O)	(+)		
Pest abundance					
Bean	♦			1	(Hansen et al., 2008)
	♦		♦	1	(Tingey and Lamont, 1988)
Chickpea	♦			3	(Das, 1998; Hossain, 2003; Mehto et al., 1988)
Chili pepper	♦			1	(Chen et al., 1995)
Cotton	♦			10	(Chen et al., 1994, 1998, Ma et al., 2006b, 2007b; Mu et al., 1993; Parajulee and Slosser, 1999; Parajulee et al., 1997; Wang et al., 2009a; Zhang, 1990; Zhao et al., 1987)
	♦		♦	2	(Wang and Zhao, 1993; Xia et al., 2000)
Mustard	♦			2	(Ansari et al., 2007; Tiwari et al., 2005)
		♦		1	(Mishra et al., 2001)
Oilseed rape		♦		3	(Hummel et al., 2009b, 2009a; Paulsen et al., 2006)
	♦			2	(Hummel et al., 2010; Sarwar, 2011)
Pea	♦			1	Ndzana et al. 2014Ndzana et al. 2014(Ndzana et al., 2014a)
Sorghum	♦	♦		1	(Phoofolo et al., 2010)
Soybean	♦			2	(Hammond and Jeffers, 1990; Miklasiewicz and Hammond, 2001)
Sugarcane	♦	♦		1	(Masih et al., 1988)
Wheat	♦			15	(Dong et al., 2012; Ehsan-Ul-Haq and Van Emden, 2003; Fathi et al., 2013; Ma et al., 2007a; Saeed et al., 2013; Sherawat et al., 2012; Wang et al., 2011a, 2008, 2009c, Xie et al., 2012b, 2012c, Zhou et al., 2009c, 2009d, 2013b, 2013a)
Wheat and alfalfa	♦			1	(Skelton and Barrett, 2005)
Wheat and bean	♦			1	(Yang et al., 2009b)
Wheat and pea	♦			1	(Lopes et al., 2015a)

Continuation of Table 3

Predator abundance and predation rate					
Cotton			♦	5	(Ma et al., 2007b; Parajulee et al., 1997; Wang and Zhao, 1993; Xia et al., 2000; Zhang, 1990)
		♦		2	(Chen et al., 1994; Parajulee and Slosser, 1999)
		♦	♦	2	(Ma et al., 2006b; Wang et al., 2009a)
Oilseed rape	♦		♦	1	(Hummel et al., 2012)
Sorghum		♦		1	(Phoofolo et al., 2010)
Wheat			♦	8	(Dong et al., 2012; Fathi et al., 2013; Wang et al., 2011a, 2009c; Xie et al., 2012b; Zhou et al., 2009d, 2013b, 2013a)
		♦	♦	2	(Sherawat et al., 2012; Wang et al., 2008)
		♦		1	(Saeed et al., 2013)
Wheat and alfalfa	♦			1	(Skelton and Barrett, 2005)
Wheat and pea	♦			1	(Lopes et al., 2015a)
Parasitoid abundance and parasitism rate					
Cotton		♦		1	(Chen et al., 1994)
			♦	2	(Ma et al., 2006b, 2007b)
Oilseed rape	♦	♦		1	(Hummel et al., 2010)
Wheat		♦		1	(Dong et al., 2012)
		♦	♦	1	(Wang et al., 2008)
			♦	8	(Ehsan-Ul-Haq and Van Emden, 2003; Ma et al., 2007a; Wang et al., 2011a, 2009c; Xie et al., 2012b; Zhou et al., 2009d, 2013b, 2013a)

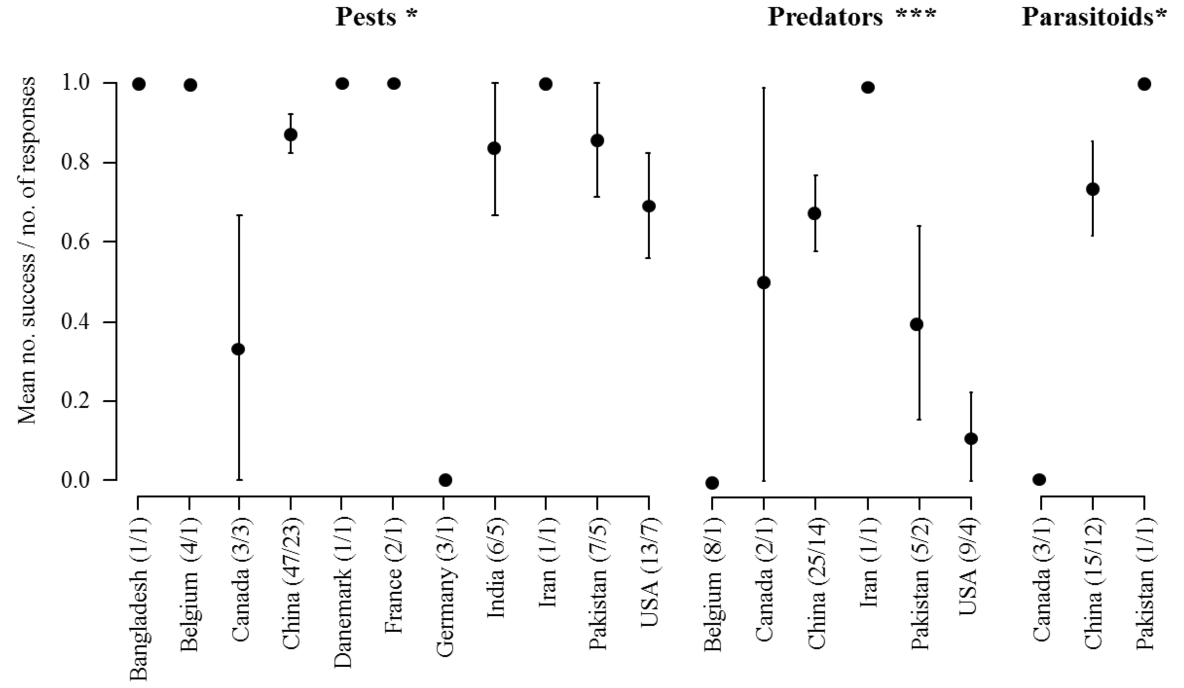

Figure 1 Mean (± SE) number of responses reporting a positive effect of wheat-based intercropping on biological control (i.e. decrease in pest and increase in natural enemy populations) on the total number of responses according to the countries where the studies took place. The ratio given in brackets corresponds to the number of responses/number of papers. Likelihood ratio tests on GLMs. * P <0.05; *** P <0.001.

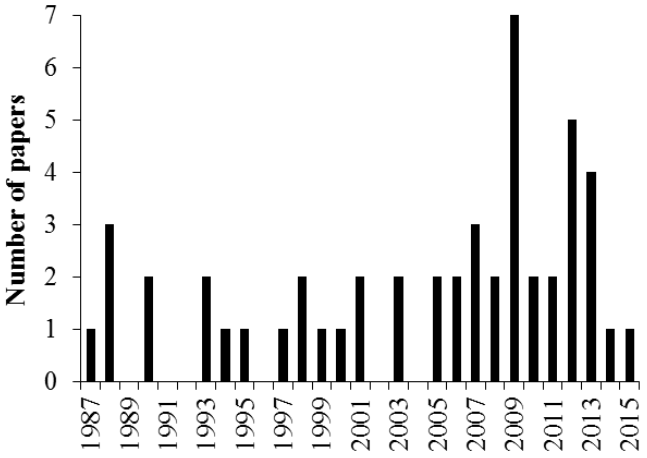

Figure 2 Evolution through time of the number of papers published on the effect of wheat-based intercropping on pests and their natural enemies.

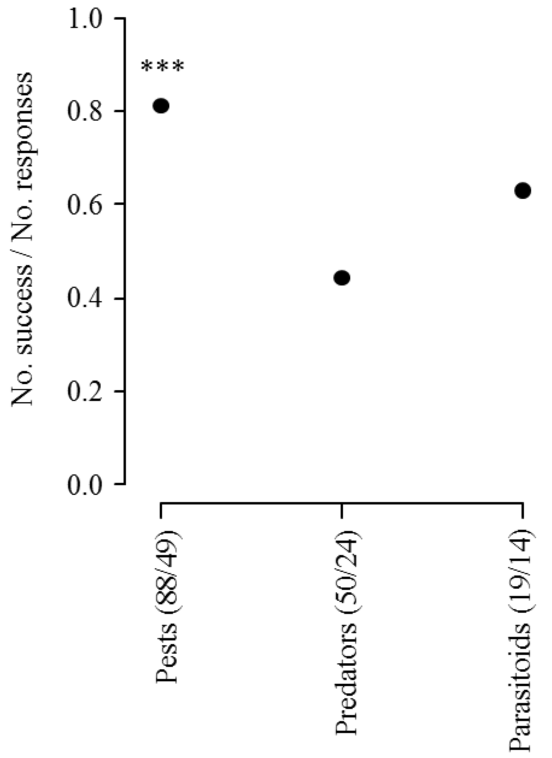

Figure 3 Ratio of the number of responses reporting a positive effect of wheat-based intercropping on biological control (i.e. decrease in pest and increase in natural enemy populations) on the total number of responses. The ratio given in brackets corresponds to the number of responses/number of papers. Exact Bernouilli test. *** P <0.001.

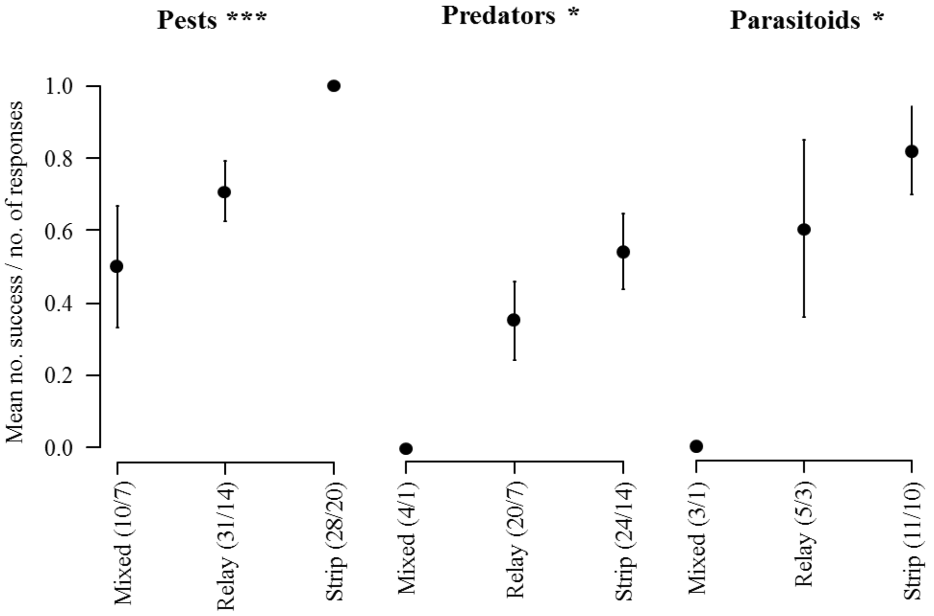

Figure 4 Mean (± SE) number of responses reporting a positive effect of wheat-based intercropping on biological control (i.e. decrease in pest and increase in natural enemy populations) on the total number of responses according to the type of wheat-based intercropping. The ratio given in brackets corresponds to the number of responses/number of papers. Likelihood ratio tests on GLMs. * P <0.05; *** P <0.001.

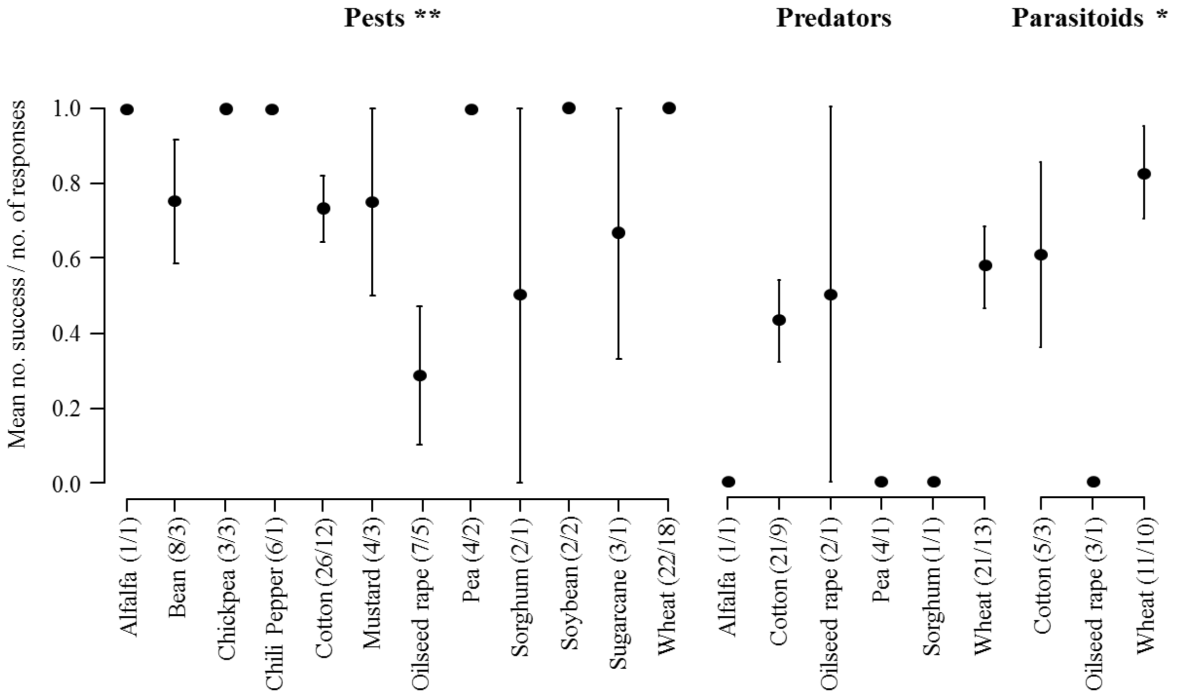

Figure 5 Mean (±SE) number of responses reporting a positive effect of wheat-based intercropping on biological control (i.e. decrease in pest and increase in natural enemy populations) on the total number of responses according to the crop species that was studied. The ratio given in brackets corresponds to the number of responses/number of papers. Likelihood ratio tests on GLMs. * P <0.05; ** P <0.01.

Chapter III Objectives and thesis structure

The review shows that wheat-based intercropping systems allow reducing pest occurrence on crops, while natural enemies are not favoured in such systems when compared to pure stands. However these results varied significantly depending on the countries where the study took place, the type of intercropping and the crops studied. In Belgium and China, more research is needed to better assess the potential of wheat-based intercropping for pest control.

Objective: Combining semiochemical with intercropping as biological control devices, and attractive natural enemies to control the number of aphids.

The present PhD thesis was realised in a goal to develop a nice integrated pest management (IPM) strategies, we use semiochemical slow-release formulations combines intercropping and protein elicitors in order to attract predators and parasitoids of aphids to control their populations on crops. This challenging work was achieved considering various questions as presented hereafter:

- *How to isolate crop intercropping with wheat on the control of aphids?*
- *Which formulate semiochemicals in slow-release devices have an efficient to attract aphid predators and parasitoids?*
- *How to use semiochemicals combine with intercropping to maximize pest control in China and Belgium?*

We attempted to answer the first question of this thesis in **Chapter Ⅲ**. The review based on fifty original research papers, the findings show that other practices could be combined with intercropping to favour natural enemies and enhance pest control.

Before presenting the results and the answers to the previous questions, a brief research was conducted in order to have an overview of the intercropping on the spread of aphids, to assess the potential impact of intercropping systems attracting natural enemies on the virus transmission. The research is presented in **Chapter IV**. After make sure that the intercropping can reduce the dispersal of aphids in the presence of predators, *in fine* potentially limiting virus dispersal, especially shortly after aphids colonize plants.

Next step will be to solve how to increase the number of natural enemies of intercropping in Belgium and China. In **Chapter V.1**, in Belgium, a two-year (2015, 2016) setup involving wheat-pea strip intercropping combined with the release of E-β-farnesene (EBF) or methyl salicylate (MeSA) was tested as a push–pull strategy to simultaneously repell aphids and attract beneficials. In **Chapter V.2**, one year (2016) setup involving wheat-pea strip intercropping combined with the release of EBF) or MeSA was tested as a push-pull strategy to simultaneously repell aphids and attract beneficials.

General discussion and conclusion are presented in **Chapter VI**. The last chapter **Chapter VII** presents all the scientific productions realised during this PhD thesis.

Chapter IV Anti-predator behaviour in intercropping system

Introduction to Chapter IV

In **Chapter Ⅲ**, fifty original research papers were obtained from a systematic search of the peer-reviewed literature. In the majority of studies, pest abundance was significantly reduced in intercropping systems compared with pure stands. However, the occurrence of their natural enemies as well as predation and parasitism rates were not significantly increased. Therefore, other practices could be combined with intercropping to favour natural enemies and enhance pest control.

In fact, aphids are known to invest in costly antipredator behavior when perceiving cues of predators. Dispersal of viruses is intimately tied to their vectors. Before presenting the results and the answers to the previous questions, in **Chapter IV**, a brief research was conducted in order to have an overview of the intercropping on the spread of aphids, to assess the potential impact of intercropping systems attracting natural enemies on the virus transmission.

Antipredator response of aphids to ladybeetles: effect of intercropping on aphid dispersal

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Abstract

Dispersal of viruses is intimately tied to their vectors. Aphids are known to invest in costly antipredator behavior when perceiving cues of predators. It is hypothesized that the absconding behavior of aphids in the presence of predators can increase virus spread in fields. Whereas most of the studies investigating this hypothesis were conducted in monoculture, we studied aphid antipredator behavior in intercropping with wheat (*Triticum aestivum* L.)-broad bean (*Vicia faba* L.) as a model. The bird cherry-oat aphid, *Rhopalosiphum padi* Linnaeus (Hemiptera: Aphididae), is an important vector of the barley yellow dwarf virus. The effects of two natural aphid enemies, adult and larvae of the seven-spot ladybeetle, *Coccinella septempunctata* Linneaus (Coleoptera: Coccinellidae), on *R. padi* dispersion was studied under laboratory conditions. Trays composed of 7 × 8 lines of plants were considered. In intercropping, one line of broad-bean succeeded one line of wheat. Six treatments were compared: in both wheat monoculture and intercropping, aphids were introduced alone, with ladybeetle larvae or with ladybeetle adults. Aphids and predators were introduced on wheat tillers in the middle of the system (source line) and aphids were counted on every plants after two and 24 hours. Results show that the total number of aphids was higher in intercropping than monoculture in treatments without ladybeetles, while the contrary was observed in the presence of ladybeetle larvae. But after 24 hours, such differences were not observed anymore. However, in receptor lines (other lines than the source one), two hours after the experiment started, aphids were more abundant in monoculture than intercropping in the presence of ladybeetle adults and larvae and after 24 hours, it was still the case in the presence of predatory larvae. These results might be explained by the non-host plant chemical cues and the physical barrier that was broad-bean plants confusing *R. padi* when searching for their host plants after being dropped from wheat by predators (i.e. associational resistance). This study shows that intercropping can reduce the dispersal of aphids in the presence of predators, *in fine* potentially limiting virus dispersal, especially shortly after aphids colonize plants.

Key Word: Vectors, Predators, *Rhopalosiphum padi*, *Coccinella septempunctata*, Intercropping, Associational resistance

1 Introduction

Aphids (Hemiptera: Aphididae) damage crops by feeding on phloem sap, while some species are efficient vectors of viruses (Goggin, 2007). Chemical insecticides, used to control them, constitute a danger for the environment (Wu and Guo, 2003). Moreover, because of their recurrent applications overtime, aphids develop resistances, rendering insecticides less effective (Bass et al., 2014; Foster et al., 2014; Lu and Gao, 2009). Hence, there is a need of exploring alternative strategies for managing pest populations. A substantial body of literature has illustrated that insect pests are less problematic in areas with an increased plant species diversity (Letourneau et al., 2011a; Malézieux et al., 2009b). Intercropping systems for instance, which consist in cultivating at least two plant species simultaneously in the same field without necessarily being sown or harvested at the same time (Lithourgidis et al., 2011a; Xie et al., 2012a), can be less sensitive to aphid populations compared with monocultures (Labrie et al., 2016; Lopes et al., 2016; Wang et al., 2009b). Additionally, intercropping cereal with leguminous crops, e.g. wheat (*Triticum aestivum* L.) with broad bean bean (*Vicia faba* L.), allows reducing nitrogen inputs, favoring the adoption of such a practice by farmers (Gooding et al., 2007; Li et al., 2009; Tosti and Guiducci, 2010; Xiao et al., 2004; Yang et al., 2009a).

Aphids are vectors of viruses and their population dynamics play a key role in the dispersal of vector-borne plant viruses. Natural enemies affect vectors by causing direct mortality, but can also promote their dispersion because of their prey's induced antipredator behavior (Dill et al., 1990; Villagra et al., 2002). Aphids can respond directly to predator attacks by escaping, defending themselves or counterattacking. They can perceive predators in advance through cues associated with the presence of natural enemies and subsequently change their behavior to reduce predation risk. For instance, aphids emit an alarm pheromone in response to predator attacks that induces a dropping off behavior, and in case of prolonged exposure can enhance the production of winged offspring (Kunert et al., 2005; Minoretti and Weisser, 2000).

The oat-bird cherry aphid *Rhopalosiphum padi* L. is a key pest of cereals such as barley (*Hordeum vulgare* L.), wheat and maize (*Zea mays* L.), being an important vector of the barley yellow dwarf virus (BYDV). It is also a frequent prey for the seven-spotted ladybeetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). The antipredator behavior in wheat monoculture system of *R. padi* to coccinellids is one of the beat understand (Bailey et al., 1995; Smyrnioudis et al., 2001). To address this question on the effect of antipredator behavior on aphids spread in intercropping system, we used wheat as host plant, intercropped with broad bean, as non-host plants, the aphid *R. padi* and one of its main predators: larvae and adults of the *C. septempunctata*. Physical and chemical stimuli are used by aphids to locate their host from non-host plants (Döring, 2014) and intercropping, by potentially associating host with non-host plants, is known to complicate the search of host plants for aphids (Poveda et al., 2008b). Hence, how does intercropping affect anti-predator behavior of aphids in the presence of ladybeetles? We hypothesize that whereas ladybeetles can increase aphid - and thus virus vector - dispersion in monoculture because of prey anti-predator behavior, this dispersion in the presence of predators is reduced in intercropping systems.

2 Materials and methods

2.1 Plant material

Wheat and broad bean were grown from seed in a climate room chamber (T=25°C±1°C, RH= 60%±10%, photoperiod= 16:8h L: D) in plastic boxes (35 × 20 × 10 cm), and the soil was organic matter mixed with sand with a proportion of 3:1. Each box consisted in seven lines (5 cm between them) of eight plants (2 cm between them). The monoculture treatment consisted in only wheat tillers, while for intercropping, two lines of broad bean were sown besides the central line of wheat (Fig. 6). Each experimental unit was a 45 × 45 × 45 cm bug dorm insect cage maintained in the climate room containing the plastic box with plants. Plants were 10 day old (~18 cm tall) when insects were introduced.

2.2 Insects

Multi-clonal populations of *R. padi* were reared on wheat plants in plastic boxes (8 × 8 × 8 cm). They were kept in a controlled environment chamber (18-22ºC, 16:8h L: D) and no efforts were made to control humidity. As for ladybeetles, *C. septempunctata* were reared in cages (40 x 40 x 80 cm) placed in a controlled environment chamber (18-22°C, 16:8h L: D, 80% relative humidity) and fed with pea aphids (*Acyrthosiphon pisum* Harris) on broad bean plants. The sex of ladybeetle adults was determined according to Baungaard (1980).

2.3 Aphid dispersal

Six treatments, with four repetitions each, were conducted: wheat monoculture without ladybeetles (WW_Aphids), wheat monoculture with ladybeetle adults (WW_Aphids+LB), wheat monoculture with ladybeetle larvae (WW_Aphids+LBL), wheat-broad bean intercropping without ladybeetles (WW_BB_Aphids), wheat-broad bean intercropping with ladybeetle adults (WW_BB_Aphids+LB), wheat-broad bean intercropping with ladybeetle larvae (WW_BB_Aphids+LBL). One hundred aphids (starved one hour before starting the experiment) were placed on the wheat tillers of line I (source line, Fig. 1) in each box. As for predators, two ladybeetle larvae (3rd instar) or two ladybeetle adults (one male and one female) were also placed on the source line plants five minutes after the introduction of aphids. The experimental boxes were maintained in a climate-controlled room under the conditions explained in ‘Plant material’. The number of aphids was recorded on the source line and on the receptor lines 0.25h, 0.5h, 1h, 2h, 4h, 6h and 24h after the introduction of aphids.

2.4 Statistical analyses

First, time (i.e. two hours, 24 hours) and crop design (i.e. monoculture, intercropping) were considered separately. For each time and crop design, generalised linear models (GLM) were fitted to assess the effect of ladybeetle treatments (i.e. aphids, aphids+LB, aphids+LBL) on aphid abundance found on (i) the source line and (ii) receptor lines. GLM were tested using independent-test, and ladybeetle treatments were compared by using Duncan post-hoc test.

Second, time (two hours, 24 hours) and ladybeetle treatments (i.e. aphids, aphids+LB, aphids+LBL) were considered separately. For each time and ladybeetle treatments, GLM were fitted to assess the effect of crop design (i.e. monoculture vs. intercropping) on aphid abundance on (i) all crop lines merged, (ii) the source line only, (iii) receptor lines only. GLM were tested using independent-test.

To meet assumptions of normality and homogeneity of variances, data on the number of aphids was transformed by $\log_{10}(n+1)$, but for presentation untransformed arithmetic means and standard deviation were used. GLM and independent-tests were applied by SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3 Results

3.1 Effect of ladybeetle adults and larvae

In wheat monoculture, aphid density on the source line was not significantly different between the treatments after two hours, and it was significantly lower in the presence of ladybeetle larvae than in other treatments after 24 hours. Dispersal of aphids towards receptor plants in the treatments with ladybeetle adults and larvae was higher than in the treatment without ladybeetles two hours after the experiment started. No differences between the three treatments were observed on the receptor lines 24 hours after the experiment started.

In wheat-broad bean intercropping, after two and 24 hours, aphid density on the source line was significantly different between each treatment, being the lowest in the presence of ladybeetle larvae and the highest without predators. However, the dispersal of aphids towards receptor plants was not significantly different between the treatments after two and 24 hours (Table 4).

3.2 Effect of intercropping vs. monoculture

Two hours after the experiment started, the total number of aphids in all lines on the treatment without predators and with ladybeetle larvae were significantly different between monoculture and intercropping (**Figure 7**a, t6=-2.94, p=0.026; t6=4.72, p=0.003, respectively). Nevertheless, after 24 hours no differences were observed anymore (**Figure** 7b).

On the source line, the number of aphids without ladybeetles was significantly lower in monoculture than in intercropping after two and 24 hours (**Figure 8**a, t6=-4.62, p=0.003; Fig. 3b, t6=-3.30, p=0.016). In the presence of predators, it is only at 24 hours that aphids were significantly less abundant in monoculture than in intercropping (**Figure 8**b, t6=-24.22, p<0.001). For the other cases, no significant differences were observed.

On receptor lines after two hours, the number of aphids was significantly reduced in intercropping compared to monoculture, with ladybeetle adults and larvae (**Figure 9**a, t6=3.60, p=0.011; t6=6.69, p<0.001, respectively). After 24 hours, only in the treatment with ladybeetle adults, aphids were less abundant in intercropping than monoculture (**Figure 9**b, t6=2.63, p=0.039).

4 Discussion

The present study shows that in wheat monoculture, both ladybeetle adults and larvae promoted the dispersal of *R. padi* towards the initially uninfected plants nearby, especially shortly after the introduction of predators (i.e. two hours after the experiment started). Moreover, it shows that this dispersion was limited in intercropping systems, confirming our hypothesis.

Previously, Smyrnioudis (2001) also observed an increased dispersion of aphids in the presence of natural enemies (parasitoids in their case) in monocultures. However in our experiment, no significant differences were observed on receptor lines after 24 hours between treatments with and without ladybeetles. It indicates that in this time-frame, aphids independently from predators, were able to colonize the whole tray. Nevertheless, a reduced abundance of aphids on the source line in the presence of ladybeetle larvae was observed, which may be due to the feeding behavior of the predators, or the aphids dropping from plants (Belliure et al., 2011). As for wheat-broad bean intercropping, the absence of differences on the receptor lines between the treatments with and without ladybeetles during the whole experiment indicates that, despite the presence of predators, *R. padi* dispersal was limited.

Without predators, the number of aphids was not significantly different on the receptor lines between monoculture and intercropping. But in the presence of predators, there were significantly more aphids on the receptor lines in monoculture than in intercropping, except on the receptor lines after 24 hours with ladybeetle larvae. However with predators, they were no significant differences on the source line between monoculture and intercropping, except in the presence of ladybeetle larvae at 24 hours. It shows that intercropping limits the ability of aphids to disperse, even in the presence of predators, which may favor an increased efficiency of predation. In intercropping systems, non host plants can represent chemical and physical barriers limiting the ability aphids to find their host plants after being dropped from wheat (Lopes et al., 2015b). Predator size and foraging speed have been noticed as factors used by aphids to assess predation risks, also, the consumption rate of *C. septempunctata* larvae was much higher than the adult one (Brodsky and Barlow, 1986).

In agroecosystems, dispersal has important consequences not only in impacting the regional population dynamics, but also in impacting the epidemiology (Ward et al., 1998). The dispersal of viruses and other pathogens transmitted by arthropods is intimately tied to the dispersal of their vectors (Jeger et al., 2009). Hence, the effects of predators on vectors might affect virus spread. Understanding how intercropping affects vector populations and behavior spread would participate in assessing how such a practice may affect pathogen spread. Several studies evaluated the impact of intercropping on disease spread for vector-borne viruses. Fargette and Fauquet (1988) suggested that mixed cropping including cassava (*Manihot esculenta* Crantz) may allow decreasing whitefly (Hemiptera: Aleyrodidae) vector populations, hence the spread of cassava mosaic disease. Moreover, Fondong et al. (2002) observed that cassava intercropped with maize or cowpea (*Vigna unguiculata* L.) allows decreasing adult whitefly populations on cassava by 50% and cassava mosaic disease incidence by 20%. Therefore, we can hypothesize that intercropping can reduce the transmission of BYDV by *R. padi*. Nevertheless, such a hypothesis remains to be tested.

After dropping to the ground, aphids can incur significant mortality from desiccation (Roitberg and Myers, 1978, 1979). In the present experiment, aphids that were dislodged from the plants were able to search and find another plant in intercropping system. Nevertheless, in the field, aphids on soil could encounter many dangers, such as ground predators or infection by entomopathogenic fungi (Ramezani et al., 2013). Thus, the survival of aphids disturbed by natural enemies may be lower than in this experiment. Moreover, due to the small size of boxes and short distance between plants, predatory larvae could easily move from one plant to another. However, this moving may be reduced in field conditions.

In summary, our results show that a higher dispersal of aphids occurs in the presence of ladybeetle adults and larvae in wheat monoculture than in wheat-broad bean intercropping, and that this might be due to the non-host plant chemical and physical cues confusing *R. padi* when searching for their host plants after being dropped from wheat. This is the first time that the effect of predators on aphid spread was studied in intercropping system. Future research will need to assess whether the anti-predator behavior of *R. padi* indeed affects the spread of BYDV in intercropping systems compared to monocultures.

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Figures and Tables:

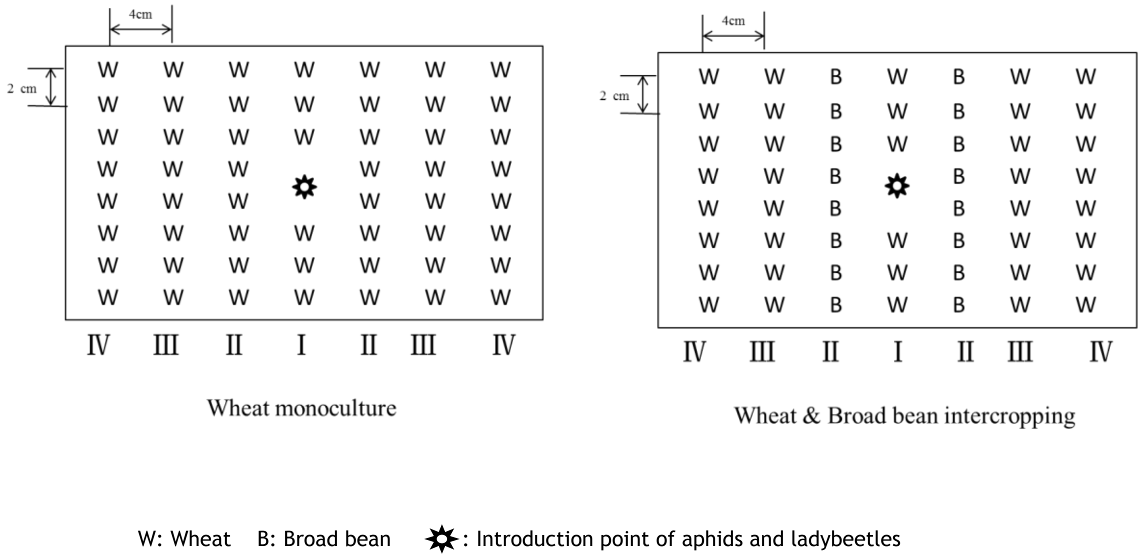

Figure 6 Design of boxes for wheat monoculture and wheat-broad bean intercropping (source line: Ⅰ, receptor lines: Ⅱ, Ⅲ, Ⅳ). Aphids and ladybeetles were introduced in the middle of the source line.

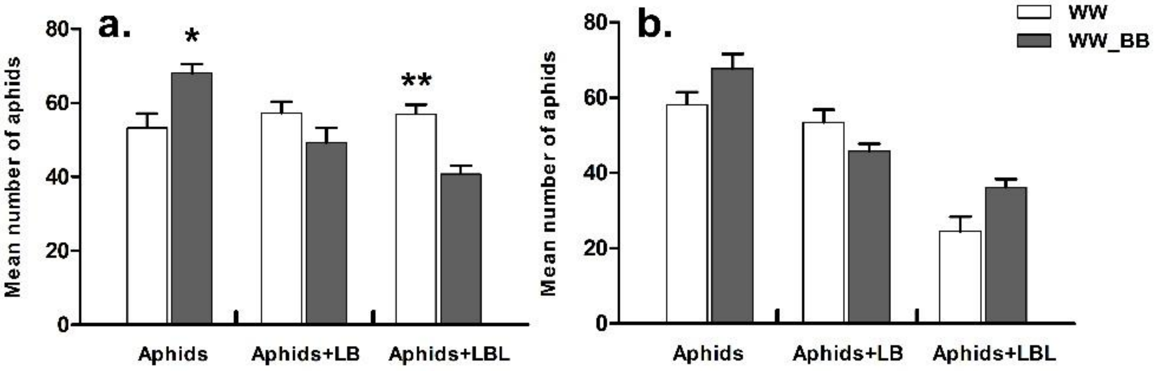

Figure 7 Mean number of aphids (± SE) on all lines (source and receptor lines) found (a) two hours and (b) 24 hours after the experiment started (LB: ladybeetle adults, LBL: ladybeetle larvae). (Independent-test, * P<0.05, ** P<0.01).

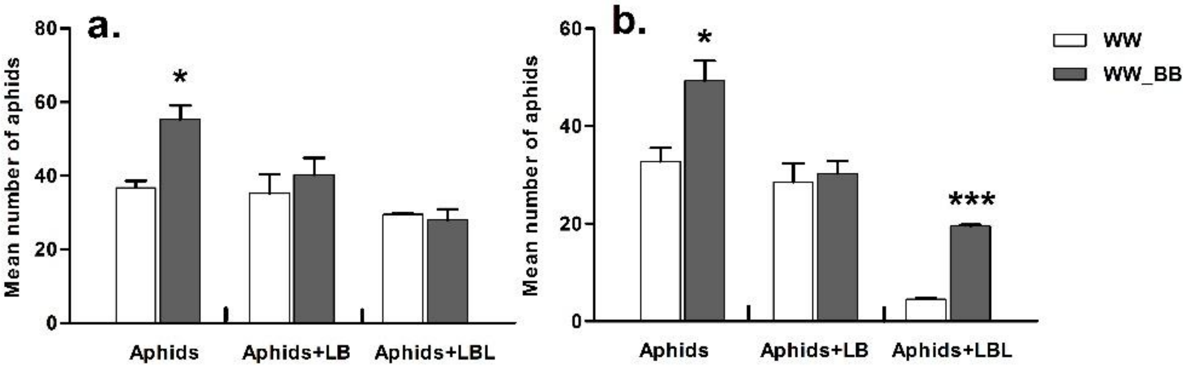

Figure 8 Mean number of aphids (± SE) on the source line found (a) two hours and (b) 24 hours after the experiment started (LB: ladybeetle adults, LBL: ladybeetle larvae). (Independent-test, * P<0.05, *** P<0.001)

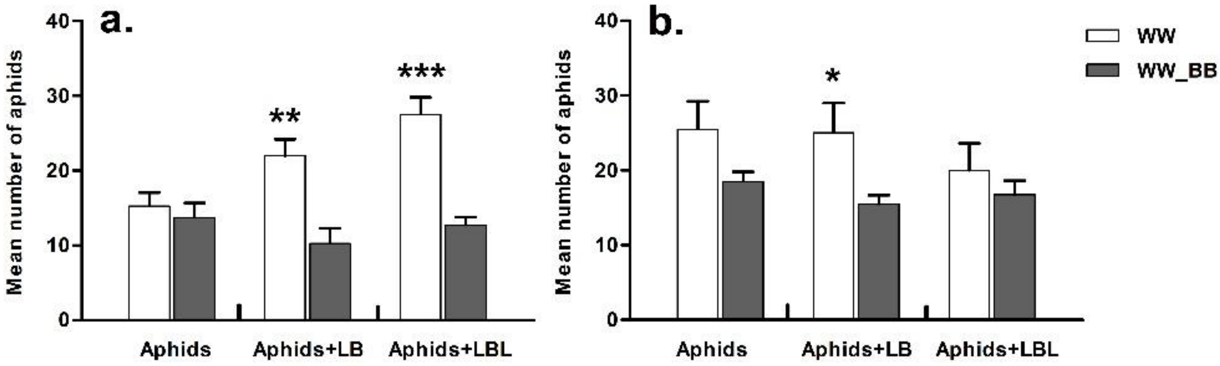

Figure 9 Mean number of aphids (± SE) on the receptor lines found (a) two hours and (b) 24 hours after the experiment started (LB: ladybeetle adults, LBL: ladybeetle larvae). (Independent-test, * P<0.05, ** P<0.01, *** P<0.001)

Table 4 Mean number of aphids (± SE) on plants (source line and receptor lines) found 2h and 24h after the aphid dispersal experiment started (LB: ladybeetle adults, LBL: ladybeetle larvae). Letters indicate significant differences based on post-hoc tests of Duncan performed on GLM (p<0.05).

		Source line		**Receptor lines**	
		2 h	**24 h**	**2 h**	**24 h**
WW	Aphids	36.75±1.89a	32.75±2.78a	15.25±1.79a	25.50±3.75a
	Aphids+LB	35.25±5.11a	28.50±3.84a	22.00±2.19b	25.00±3.98a
	Aphids+LBL	29.50±0.29a	4.50±0.29b	27.50±2.33b	20.00±3.62a
WW_BB	Aphids	55.25+3.82a	49.25±4.11a	13.75±1.93a	18.50±1.32a
	Aphids+LB	40.25±4.57b	30.25±2.56b	10.25±2.02a	15.50±1.19a
	Aphids+LBL	28.00±2.89c	19.50±0.29c	12.75±1.03a	16.75±1.89a

Chapter V Intercropping combined with semiochemicals release in Belgium and China

Introduction to Chapter V

In **Chapter IV,** the results show that the non-host plant chemical cues and the physical barrier that was broad-bean plants confusing *R. padi* when searching for their host plants after being dropped from wheat by predators. After make sure that the intercropping can reduce the dispersal of aphids in the presence of predators, *in fine* potentially limiting virus dispersal, especially shortly after aphids colonize plants. Then, we will try to solve how to increase the number of natural enemies of intercropping in Belgium and China. The weather conditions were different in Belgium and China, also the number of aphids during the wheat stage, so we did the experiment in both countries.

In **Chapter V.1**, in Belgium, a two-year (2015, 2016) setup involving wheat-pea strip intercropping combined with the release of E-β-farnesene (EBF) or methyl salicylate (MeSA) was tested as a push–pull strategy to simultaneously repell aphids and attract beneficials. Two types of slow-release formulation (i.e., oil and alginate beads) containing EBF or MeSA were deployed with intercropping. The abundance of aphids was significantly decreased, hoverfly larvae and mummified aphids increased on both pea plants and wheat tillers by the release of oil-formulated EBF and MeSA. In **Chapter V.2**, one year (2016) setup involving wheat-pea strip intercropping solely, wheat-pea strip intercropping combined with the release of EBF and MeSA were tested in the field as a push-pull strategy to simultaneously repell aphids and attract beneficials. The total number of aphids throughout the growing season was significantly decreased in treatments with releases of semiochemicals compared to intercropping solely.

Chapter V.1 Semiochemical combine with wheat-pea intercropping (In Belgium)

A push–pull strategy to control aphids combines intercropping with semiochemical releases

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QX, FF, TL, and JL conceived and designed research. SH, TL, YZ, BB, and QX conducted experiments and analyzed data. QX, SH, FF, JL, and TL wrote and modified the paper.

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Abstract

Even if insect pest populations can be reduced by increasing plant diversity through intercropping, natural enemies are not always favored in such systems. Alternatively, semiochemical substances have been tested to enhance biological control, with inconsistent results. Combining both strategies can be an interesting way to maximize pest control. In this work, a two-year setup involving wheat–pea strip intercropping combined with the release of *E*-β-farnesene (EBF) or methyl salicylate (MeSA) was tested as a push–pull strategy to simultaneously repell aphids and attract beneficials. Two types of slow-release formulation (i.e., oil and alginate beads) containing EBF or MeSA were deployed with intercropping. The abundance of aphids was significantly decreased, hoverfly larvae and mummified aphids increased on both pea plants and wheat tillers by the release of oil-formulated EBF and MeSA. The proportion parasitism of aphids-parasitism rate (mummies / [aphids + mummies]) was also increased by treating both crops in both years. Releasing EBF through oil rather than alginate beads proved significantly better for attracting natural enemies and reducing aphids. Aphids were negatively correlated with the density of hoverflies (both adults and larvae) and numbers of mummies. All these results showed that combining intercropping with the release of EBF or MeSA formulated in oil can significantly reduce aphid density and attract their natural enemies. Therefore, the combination of both strategies could help farmers reduce the use of insecticides.

Key words: *Triticum aestivum, Pisum sativum,* association, Methyl salicylate, *E*-β-farnesene, Alginate beads, Integrated pest management

1 Introduction

Integrated pest management (IPM) programs were developed because of the emergence of environmental impacts and risks for human health associated with the use of synthetic pesticides (Brewer and Goodell 2012; Hassanali et al. 2008; Hokkanen 2015). Biological pest control, one among other IPM tactics, involves using living organisms (i.e., insect predators parasitoids, or pathogens) to suppress pest populations and render them less damaging that they would otherwise be, thus reducing the need for costly chemical pesticides and associated environmental and human health concerns (Lenteren and Woets 1988; Malakar et al. 2013; Zappalà et al. 2013). A substantial body of literature has illustrated that insect pests are less problematic in areas with increased plant species diversity (Dahlin et al. 2015; Knops et al. 1999; Letourneau et al. 2011). Indeed, according to the "resource concentration hypothesis" (Root 1973), specialist herbivores are more likely to find their host plants when they are concentrated in dense or pure stands. Intercropping, i.e., the simultaneous growing of two or more species in the same field for a significant period without their necessarily being sown or harvested at the same time (Lithourgidis et al. 2011) is one way to increase plant diversity (Malézieux et al. 2009; Poggio 2005). Wheat (*Triticum aestivum* L.) is one of the main cultivated cereal crops worldwide. Wheat-based intercropping systems have been extensively studied, and their utility for controlling pests was recently reviewed (Lopes et al. 2016). Whereas pests are reported to be generally reduced in such systems, their natural enemies are not necessarily enhanced.

In addition to intercropping, the strategic deployment of semiochemicals (i.e., informative molecules used in insect–insect or plant–insect interactions) has been widely considered within various IPM programs (Heuskin et al. 2012a; Mensah et al. 2014; Rodríguez and Niemeyer 2005; Sarles et al. 2015). Laboratory and field studies have demonstrated that semiochemical releases have the potential to simultaneously repel pests and attract natural enemies (i.e., 'push–pull' plant protection strategy) (Mensah et al. 2014; Ninkovic et al. 2003; Zhou et al. 2016). For instance, MeSA is a herbivore-induced plant volatile that is repellent to *Rhopalosiphum padi* and other cereal aphids (Glinwood and Pettersson 2000; Ninkovic et al. 2003) and is attractive to aphid predators such as ladybeetles (Coleoptera: Coccinellidae; e.g., *Coccinella septempunctata*) (Saona et al. 2011; Zhu and Park 2005), lacewings (Neuroptera: Chrysopidae; e.g., *Chrysopa nigricornis*) (James 2003a), hoverflies (Diptera: Syrphidae) (Mallinger et al. 2011), and aphid parasitoids (Gordon et al. 2013; Martini et al. 2014) . Moreover, the aphid alarm pheromone, the major component of which is EBF (Francis et al. 2005), acts as a repellent for plant herbivores and attracts predatory beetles (e.g., *Harmonia axyridis*) (Francis et al. 2004; Verheggen et al. 2007), hoverflies (Verheggen et al. 2008a), lacewings (Boo et al. 1998), and parasitoids (Foster et al. 2005). To be useful in IPM, release systems must be economical, environmentally safe, and field-tested for efficacy toward targeted insects before legal authorization and commercialization. Currently, two main types of slow-release dispensers are available on the market: liquid formulations such as paraffin oil (Verheggen et al. 2008b; Zhou et al. 2016), and solid matrix dispensers such as alginate beads (Heuskin et al. 2012a; Yosha et al. 2008). To assess the effectiveness of these devices in repelling pests and attracting natural enemies, field experiments under natural conditions are needed (Daems et al. 2016).

Whereas intercropping alone may not enhance pest natural enemies, the use of semiochemicals in monocultures may not be consistently successful and may even negatively influence natural enemies in low pest density situations (Wang et al., 2011b). Combining semiochemicals with intercropping may bridge these problems. Particular attention has been paid to intercropping wheat with pea (*Pisum sativum* L.) as a way to reduce nitrogen inputs (Bedoussac and Justes 2010; Hauggaard-Nielsen et al. 2008). Moreover, wheat–pea strip intercropping was reported to decrease pea aphid *Acyrthosiphon pisum* Harris and *Sitobion avenae* Fabricius populations when compared with pure stands, although aphid natural enemies were not particularly attracted (Lopes et al., 2015b; Ninkovic et al., 2003; Seidenglanz et al., 2011; Zhou et al., 2009a). In this context, the aims of this study were to: (i) determine if combining wheat-pea strip intercropping with the release of EBF or MeSA can better repel aphids and simultaneously attract their natural enemies than intercropping alone and (ii) evaluate the comparative efficacy of two slow-release formulations of EBF (i.e., oil and alginate beads).

2 Materials and methods

2.1 Field layout

This study was conducted at the experimental farm of Gembloux Agro-Bio Tech, University de Liège, Namur Province of Belgium (50°30' N, 4°43' E) in 2015 and 2016. Four treatments were tested: (1) wheat-pea strip intercropping (control), (2) wheat-pea strip intercropping with EBF release using alginate beads (WP_EBF bead), (3) wheat-pea strip intercropping with EBF release formulated in oil (WP_EBF oil), and (4) wheat-pea strip intercropping with MeSA release formulated in oil (WP_MeSA). Each treatment was repeated four times, resulting in a Latin square design made of 16 80 m^2 plots (10m x 8m) (Fig. 10).

Each plot was composed of three strips of spring wheat (variety 'Tybalt', 350 seeds/m^2) and two strips of spring pea (variety 'Kayanne', 35 seeds/m^2), each strip being 2 m long. Wheat and pea were sown on 13 March 2015 and 15 March 2016. No insecticides or herbicides were used in the experimental area.

2.2 Weather conditions

Temperature was measured using a data logger (EASYLOG USB-2) placed in the experimental field, whereas rainfall data were taken from a meteorological station near the experimental farm (Bordia, Gembloux, 50°56’N, 4°71’E). Mean temperatures were similar for each month of the experiment in 2015 and 2016, but rainfall was two to three times higher in 2016 (Table 5).

2.3 *E*-β-farnesene and methyl salicylate dispensers

EBF was formulated in paraffin oil at a concentration of 10mg/mL, whereas EBF was formulated in alginate beads (6% w/v) and prepared using the methodology of Heuskin et al. (2012b). MeSA (ReagentPlus®, a99%) was purchased from Sigma Aldrich (Bornem, Belgium). For the experiment, 200 mg of EBF beads (6% w/v) were placed in a permeable net bag (6 × 6 cm) that was fixed to a trap stake in each WB_EBF bead treatment plot and 100 μL of EBF oil (10mg/mL) was placed in a 1 cm-diameter rubber septum that was fixed to a trap stake in each WP_EBF oil plot. Finally, 200 μL pure MeSA was placed in a 1 cm-diameter rubber septum and fixed to a trap stake in each WP_MeSA plot. All release devices were placed under a plastic roof (35 × 35 cm) to protect them from rain and changed every seven days, except EBF beads were replaced every 35 days.

According to previous studies, around 500 μg of EBF is released from 100 mg EBF alginate beads over 35 days and around 100 μg of EBF is released from 100 μL EBF oil over seven days in laboratory-controlled conditions (20 ℃, 65% relative humidity, airflow 0.5 L min^{-1}) (Heuskin et al. 2012b; Zhou et al. 2016). This information was used to calculate similar EBF daily release rates for field experiments. According to James (2003), about 210 mg of MeSA was needed over seven days to obtain significant results, which would translate to about 234 mg MeSA released in our experiment. The first application of semiochemicals was on the 28 May in 2015 and 13 May in 2016.

2.4 Sampling of aphids and beneficials

Aphids (all instars), aphid predators (i.e., ladybeetle, hoverfly, and lacewing larvae), and mummified aphids were counted on pea plants and wheat tillers every seven days from the 28th of May to 29th of July 2015 and 19th of May to 22nd of July 2016. In both wheat and pea crops, a series of 10 pea plants or wheat tillers were randomly selected at each of four locations in each plot. Aphids were identified to species level based on morphological characters described by Blackman and Eastop (2008).

Alate aphids, adult ladybeetles, hoverflies and lacewings were collected using yellow pan traps (Flora®, 27 cm diameter and 10 cm depth). Traps were attached to fiberglass stakes, positioned at crop height, and filled with water and few drops of detergent to reduce water surface tension. A single trap was placed in the middle of each plot ($n = 16$ total traps). Traps were emptied and refilled weekly during the same periods in 2015 and 2016. Trap contents were decanted through a 0.5 mm mesh sieve, and collected insects were transferred to plastic vials containing 70% ethanol. All individuals were identified in the laboratory to species level, using specific identification keys: Taylor (1981) for aphids, Roy et al. (2013) for ladybeetles, Veen (2010) for hoverflies, and San Martin (2004) for lacewings. The number of individuals of each species was recorded.

2.5 Statistical analysis

The effects of treatments and years (i.e., explanatory variables) on the abundance of both observed and trapped insects were first analyzed by fitting generalized linear mixed models (GLMM, package lme4, Bates et al. 2014) with Poisson error distribution (log-link function). Explanatory variables and their interactions were included as fixed effects, whereas the sampling dates were introduced as random ones. The effects of fixed factors were tested using likelihood-ratio tests ($p < 0.05$). Second, analyses of variance ($p < 0.05$) were used to assess the effects of these same variables on the proportional parasitism of aphids (mummies / [aphids + mummies]). Tukey's test was used to separate treatment means ($p < 0.05$). When interactions were significant, the effect of treatment was assessed separately for each year. Finally, linear regressions were used to analyze the relationship between aphid and natural enemy abundance. For each insect taxon, total abundance over the sampling period for each year separately was summed, considering each repetition in each treatment, then $\log_{10}(n+1)$-transformed prior to analysis. All analyses were performed using R 2.6.2 (R Core Team, 2013b).

3 Results

3.1 Aphid and natural enemy diversity

One species of aphid was observed on pea plants (*A. pisum*) and three on wheat tillers (*Metopolopium dirhodum* (Walker), *S. avenae* and *R. padi*). Species richness was highest for hoverflies (nine species), followed by ladybeetles (three species) and lacewings (one species) (Table 6).

3.2 Observations on plants

Aphid abundance on both pea plants and wheat tillers was affected by treatment in both years (pea in 2015: $\chi^2 = 241.4$, df = 3, $p < 0.001$; pea in 2016: $\chi^2 = 151.5$, df = 3, $p < 0.001$; wheat in 2015: $\chi^2 = 26.8$, df = 3, $p < 0.001$; wheat in 2016: $\chi^2 = 292.5$, df = 3, $p < 0.001$), as was the abundance of hoverfly larvae and mummified aphids (Table 7). The proportion of aphids parasitized was also affected by treatment on both crops in both years (pea in 2015: $F = 46.1$, df = 3, $p < 0.001$; pea in 2016: $F = 47.2$, df = 3, $p < 0.001$; wheat in 2015: $F = 29.2$, df = 3, $p < 0.001$; wheat in 2016: $F = 27.9$, df = 3, $p < 0.001$), but the effect of year was significant only for pea (Table 7).

Aphid abundance was significantly lower in both years on pea and wheat in the WP_EBF oil and WP_MeSA treatments compared to control (Fig. 11a, 12a). Moreover, hoverfly larvae and pupae were more abundant in the WP_MeSA treatment for both pea plants and wheat tillers in both years (Fig. 11b, 12b), whereas a higher proportion of aphids were parasitized in both WP_EBF oil and WP_MeSA treatments compared to controls (Fig. 11c, 11d, 12c, 12d). The effect of the WP_EBF bead treatment was significant only for proportion of aphids parasitized on pea in both years, and on wheat tillers only in 2015 (Fig. 11d, 12d). Too few adult lady beetle adults and larvae and lacewing larvae were observed on either plant type to permit statistical analysis.

3.3 Trapped aphids and adult predators

Catches of winged wheat and pea aphids, as well as adult ladybeetles, lacewings, and hoverflies, were significantly affected by treatment (Table 8). Only lacewings were affected by the experimental year. Pea aphids were significantly reduced in the WP_MeSA treatment compared to control (Fig. 13a), whereas results for the WP_MeSA treatment were different for wheat aphids across years (Fig. 13b). Ladybeetles were significantly higher in the WP_EBF oil treatment than in all other plots in both years (Fig. 13c), whereas hoverflies were significantly higher in the WP_EBF oil and WP_MeSA treatments compared to control (Fig. 13d). Different results were found across years for lacewings, the WP_MeSA treatment attracting more in 2015, but the WP_EBF oil treatment attracting more in 2016 (Fig. 13e). Nevertheless, their low abundance in 2016 was notable.

3.4 Aphid-natural enemy regressions

In general (except for aphids observed on wheat tillers in 2015), aphids were negatively correlated with densities of hoverflies (both adults and larvae) and mummies (Table 9). Inconsistent results among years were found for ladybeetles and lacewings.

4 Discussion

Release of EBF or MeSA from a paraffin oil formulation can significantly reduce aphid density and attract aphid natural enemies in intercropped agroecosystems, possibly due to two factors. The semiochemicals EBF and MeSA are repellent to aphids, but are also known to induce the development of wings, an effect that would accelerate aphid dispersal (Hatano et al. 2010; Kunert et al. 2005; Ninkovic et al. 2003; Thieme and Dixon 2015). Secondly, the natural enemies attracted by these compounds probably preyed on and parasitized the aphids, reducing their populations. Hoverflies, which were the most abundant aphid natural enemies, were positively attracted by the semiochemicals and were negatively correlated with aphid abundance. Previously, Verheggen et al. (2008) showed that EBF is an important olfactory cue for aphid location by hoverflies and that it induces oviposition, whereas Francis et al. (2005) showed EBF to be an important olfactory cue for aphid location by hoverfly larvae. As for , the present results for MeSA are consistent with those of James (2003) who observed it to attract hoverfly adults in the field. EBF was also found to be attractive to adult lady beetles, which is consistent with previous studies on several species (Cui et al. 2012; Francis et al. 2004; Leroy et al. 2012). Nevertheless, the lack of an effect of MeSA on lady beetle recruitment contradicts the results of previous field studies (Rodriguez-Saona et al. 2011; Zhu and Park 2005). However, MeSA did attract lacewings, as found by James (2003a, 2006), who studied several lacewing species including *Chrysopa nigricornis* and *Chrysopa oculata* (Neuroptera: Chrysopidae). Nevertheless, our results were inconsistent among years, lacewings being scarce in the second year when compared to the first. Moreover, almost no larvae were observed, which was the case in several previous studies conducted in the same area (Alhmedi et al. 2007, 2009; Hatt et al. 2016), calling into question their ability to control important aphid populations in the field. Finally, even though parasitoids were not caught in traps, the proportion of aphids mummified was higher in every treatment with MeSA or EBF formulated in paraffin oil compared to control. Contrasting results have been reported regarding the attraction of aphid parasitoids to MeSA and EBF (Beale et al. 2006; Du et al. 1998; Gonzales et al. 1999) and their effect on aphid parasitism in the field has been poorly studied as yet. The present results suggest that releasing such semiochemicals in fields can enhance the host-finding ability of aphid parasitoids and result in improved pest control.

The release of EBF from oil rather than alginate beads was significantly more effective in attracting natural enemies and reducing aphid densities. Some of this difference could be explained by differential sensitivity of release formulations to climatic conditions. Although alginate beads have been successfully used to release semiochemicals in field conditions, the amount released can be limited by temperature, relative humidity and, potentially, wind speed. Diffusion of semiochemicals from alginate beads has been reported to be limited by high relative humidity (>85%) (Daems et al. 2016; Heuskin et al. 2012a). Thus, the diffusion of an effective amount of compound is not guaranteed throughout the season in regions like Belgium where the weather is highly variable (note the difference in rainfall between 2015 and 2016). Whereas our results support oil to be more effective in such regions, further studies might test alginate beads in controlled environments such as greenhouses.

Increasing crop diversity through intercropping can enhance 'associational resistance' to aphids (Ndzana et al. 2014) and the addition of semiochemical releases can augment crop protection further by enhancing natural enemies while simultaneously repelling aphids, thus strengthening biological pest control. In the present study, pea aphids peaked earlier than wheat aphids in both years (17, June 2015 and 3, June 2016 vs, 25, June 2015 and 10, June 2016). By associating these two crops in the same field, natural enemies can quickly move from pea to wheat and effectively control pests on both crops. Wheat–pea association has also been promoted to reduce chemical fertilizer applications, thanks to the ability of legumes to fix atmospheric nitrogen and make it available for cereals (Bedoussac et al. 2015). The development of and intercropping-based IPM strategy, combined with the release of semiochemicals to manipulate insect behavior, should increase farmer acceptance of intercropping to reduce their use of external inputs. Whereas technical issues need to be solved for engaging the transition (Lopes et al. 2016), it could provide economic benefits while at the same time reducing negative environmental and health effects for farmers and society as a whole.

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Figures and Tables:

Table 5 Monthly means of meteorological data from Gembloux, Belgium, during the experimental periods in 2015 and 2016. Temperature was measured using a data logger (EASYLOG USB-2) placed in the experimental field. Rainfall data were taken from a meteorological station set near the experimental farm (Bordia, Gembloux, 50°56'N, 4°71'E)

	2015			**2016**		
	May	**June**	**July**	**May**	**June**	**July**
Temp (℃)	13.01	17.73	19.87	14.21	17.28	20.26
Rain (mm)	20.61	57.15	37.01	72.44	138.46	65.57

Table 6 Diversity and abundance of aphids and their trapped natural enemies

Order: Family	**Species**
Aphids (Hemiptera: Aphididae)	*Acyrthosiphon pisum* (Harris)
	Metopolophum dirhodum (Walker)
	Sitobion avenae (Fabricius)
	Rhopalosiphum padi(Linnaeus)
Ladybeetles (Coleoptera: Coccinellidae)	*Harmonia axyridis* (Pallas)
	Coccinella septempunctata Linnaeus
	Propylea 14-punctata (Linnaeus)
Hoverflies (Diptera: Syrphidae)	*Episyrphus balteatus* (De Geer)
	Melanostoma mellinum (Linnaeus)
	Eupeodes corollae (Fabricius)
	Melanostoma scalare (Fabricius)
	Eupeodes luniger (Meigen)
	Sphaerophoria scripta (Linnaeus)
	Syrphus ribesii (Linnaeus)
	Syrphus vitripennis (Meigen)
	Platycheirus peltatus (Meigen)
Lacewings (Neuroptera: Chrysopidae)	*Chrysoperla carnea* (Stephens)

Table 7 Effect of treatments (control, WP_EBF bead, WP_EBF oil, and WP_MeSA), years (2015, 2016), and their interaction on the abundance of aphids, mummified aphids, hoverflies (larvae and pupae) and parasitism rate. Degrees of freedom (df), $\chi 2$-values, and p-values from likelihood-ratio tests performed on the GLMMs are given, F-values, and p-values from analyses of variance are given. * $p < 0.05$, ** $p < 0.01$, *** $P < 0.001$

	Observations on pea			Observations on wheat		
Source of variation	**d.f.**	**χ^2**	**p-value**	**d.f.**	**χ^2**	**p-value**
Aphids						
Treatment	3	378.93	<0.001***	3	44.32	<0.001***
Year	1	346.3	<0.001***	1	514.04	<0.001***
Treatment*year	3	13.98	0.002**	3	9.77	0.02*
Mummified aphids						
Treatment	3	63.14	<0.001***	3	9.18	0.03*
Year	1	654.28	<0.001***	1	7.33	<0.001***
Treatment*year	3	7.14	0.06	3	1.34	0.72
Hoverfly larvae and pupae						
Treatment	3	21.94	<0.001***	3	17.31	<0.001***
Year	1	25.16	<0.001***	1	31.99	<0.001***
Treatment*year	3	0.38	0.94	3	0.95	0.81
Parasitism rates						
Treatment	3	57.62	<0.001***	3	47.01	<0.001***
Year	1	1009.52	<0.001***	1	4.13	0.05 ns
Treatment*year	3	36.77	<0.001***	3	9.2	<0.0012***

Table 8 Effect of treatments (control, WP_EBF bead, WP_EBF oil, WP_MeSA) and years (2015, 2016) on the abundance of winged aphids and related beneficials collected in yellow pan traps. Degrees of freedom (df), χ^2-values, and p-values from likelihood-ratio tests performed on the GLMMs are given, * $p < 0.05$, ** $p < 0.01$, *** $P < 0.001$

Source of variation	**d.f.**	**χ^2**	**p-value**
A.pisum			
Treatment	3	21.2	<0.001***
Year	1	3.39	0.07
Treatment*year	3	1.42	0.7
Wheat aphids			
Treatment	3	21.98	<0.001***
Year	1	2.57	0.11
Treatment*year	3	3.58	0.31
Lady beetles			
Treatment	3	27.2	<0.001***
Year	1	0.18	0.67
Treatment*year	3	0.19	0.98
Lacewings			

	Treatment	3	12.14	0.006**
	Year	1	308.23	<0.001***
	Treatment*year	3	4.47	0.21
Hoverflies				
	Treatment	3	79.67	<0.001***
	Year	1	1.23	0.27
	Treatment*year	3	3.02	0.39

Table 9 Linear regressions between the abundances of aphids, predators (adults and larvae) and mummies without distinguishing treatments, * p < 0.05, ** p < 0.01, *** P < 0.001

	2015				**2016**			
	Estimate	**R^2**	**F_{1-14}**	**p-value**	**Estimate**	**R^2**	**F_{1-14}**	**p-value**
Observation on the pea								
Hoverfly larvae	-1.83	0.84	71.5	< 0.001 ***	-1.35	0.25	4.74	0.047 *
Mummies	-1.5	0.51	14.6	0.002 **	-1.26	0.35	7.67	0.015 *
Observation on the wheat								
Hoverfly larvae	-0.21	0.06	0.93	0.35	-0.22	0.38	8.55	0.011 *
Mummies	-0.34	0.17	2.83	0.115	-0.31	0.3	5.89	0.029 *
In traps (pea aphids)								
Ladybeetle adults	-0.41	0.07	1.03	0.328	0.11	0.01	0.21	0.658
Hoverfly adults	-0.72	0.53	15.8	0.001 **	-0.4	0.72	35.7	< 0.001 ***
Lacewing adults	-0.8	0.55	16.9	0.001 **	-0.23	0.04	0.66	0.431
In traps (wheat aphids)								
Ladybeetle adults	-0.93	0.33	6.91	0.019 *	-0.09	0.004	0.07	0.8
Hoverfly adults	-0.57	0.31	6.33	0.025 *	-0.61	0.77	47.5	< 0.001 ***
Lacewing adults	-0.64	0.33	6.85	0.02 *	-0.47	0.09	1.32	0.27

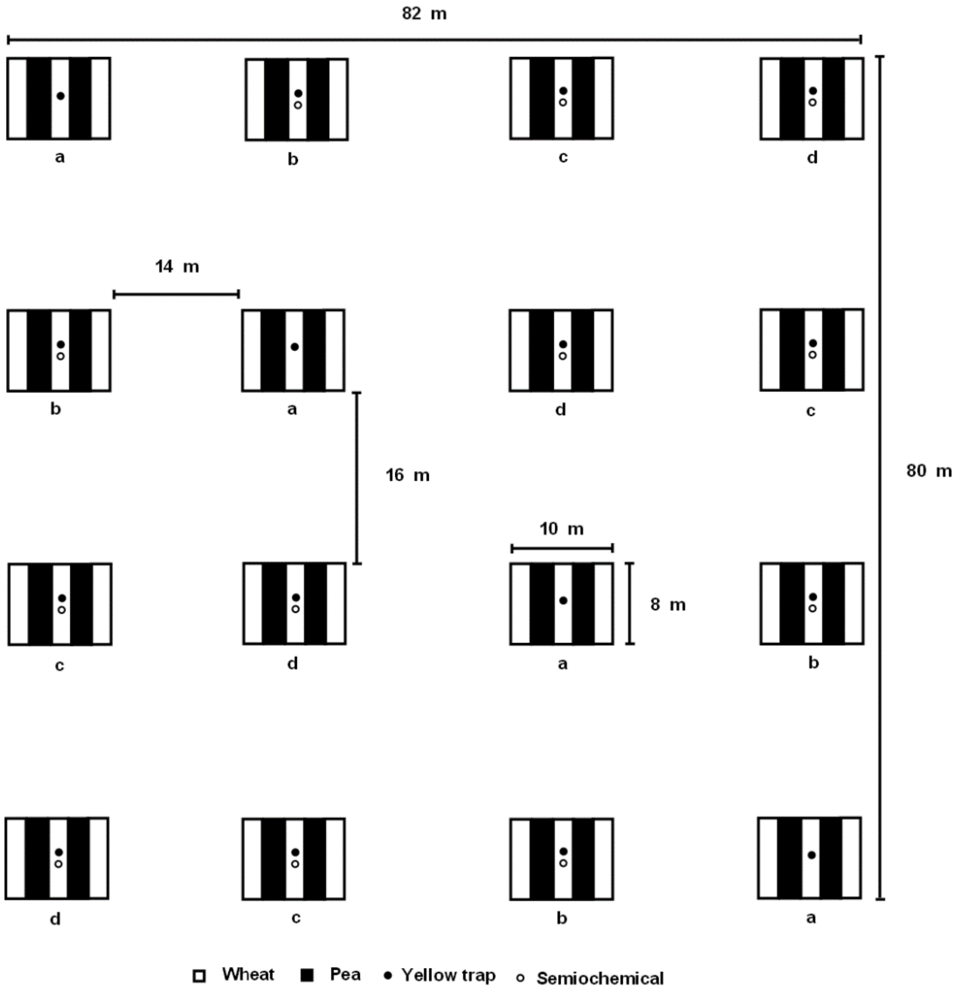

Figure 10 Experimental design (a: wheat–pea intercropping (control), b: wheat–pea intercropping with EBF release using oil (WP_EBF oil), c: wheat–pea intercropping with EBF release using alginate bead (WP_EBF bead), d: wheat–pea intercropping with MeSA release (WP_MeSA))

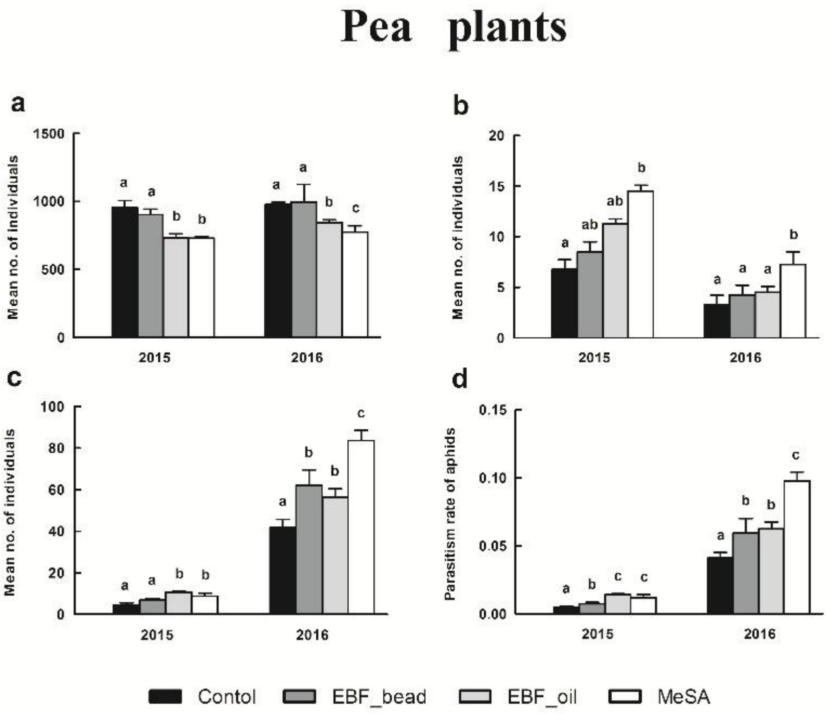

Figure 11 Mean abundance (and standard error) of a) pea aphids, b) hoverfly larvae and pupae, c) mummified aphids, and d) parasitism rate, on pea plants in each treatment (control, WP_EBF bead, WP_EBF oil, WP_MeSA) in 2015 and 2016. Letters indicate significant differences based on post-hoc tests of Tukey performed on GLMMs ($p < 0.05$)

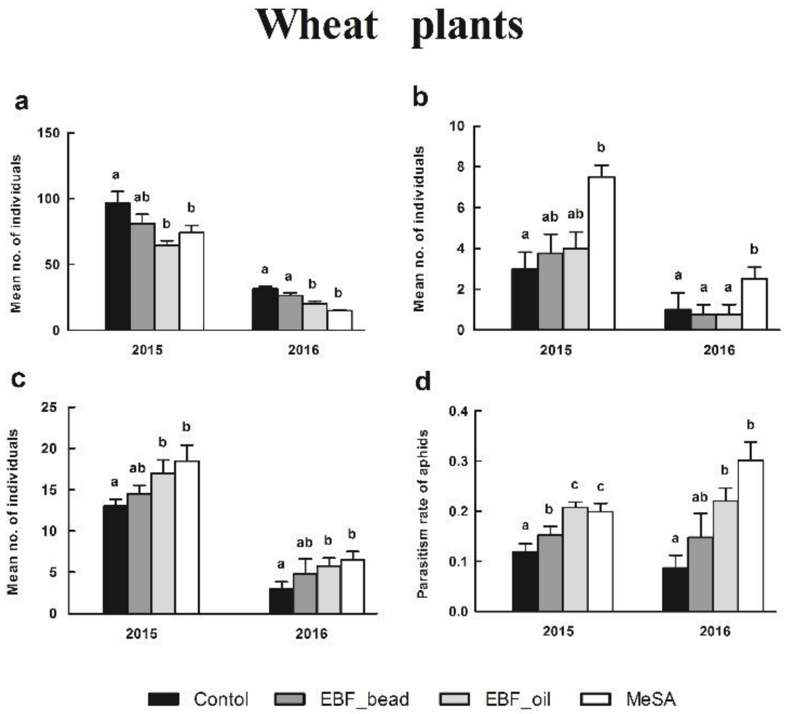

Figure 12 Mean abundance (and standard error) of a) wheat aphids, b) hoverfly larvae and pupae, c) mummified aphids, and d) parasitism rate, on wheat tillers in each treatment (control, WP_EBF bead, WP_EBF oil, WP_MeSA) in 2015 and 2016. Letters indicate significant differences based on post-hoc tests of Tukey performed on GLMMs ($p < 0.05$)

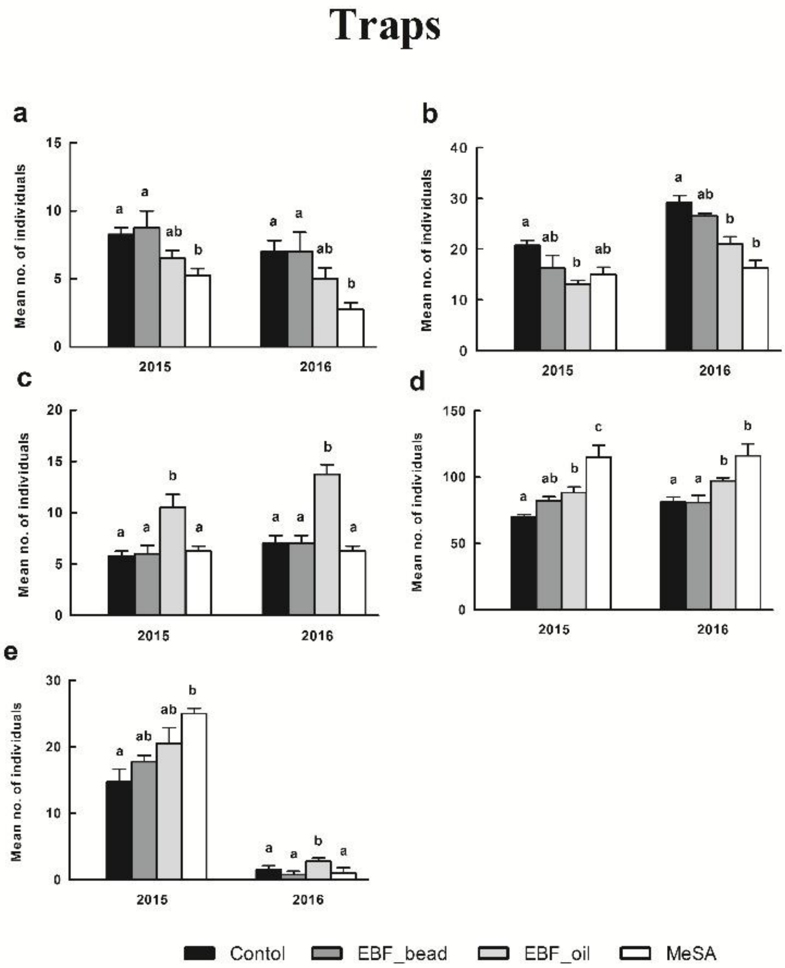

Figure 13 Mean abundance (and standard error) of a) pea winged aphids, b) wheat winged aphids, c) ladybeetle adults, d) hoverfly adults, and e) lacewing adults, in yellow pan traps in each treatment (control, WP_EBF bead, WP_EBF oil, WP_MeSA) in 2015 and 2016. Letters indicate significant differences based on post-hoc tests of Tukey performed on GLMMs ($p < 0.05$)

Chapter V.2 Semiochemical combine with wheat-pea intercropping In China

Combining *E*-β-farnesene and methyl salicylate release with wheat-pea intercropping enhances biological control of aphids in China

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Abstract

Combining intercropping with the release of semiochemicals may strengthen biological control of aphid pests as a push-pull strategy that simultaneously repels aphids and attracts their beneficials. This hypothesis was tested in China and the experiments were set-up: wheat-pea strip intercropping solely (control), intercropping combined with the release of *E*-β-farnesene (EBF), and intercropping combined with the release of methyl salicylate (MeSA), each treatment repeated three times. The total number of aphids throughout the growing season was significantly decreased in treatments with releases of semiochemicals compared to intercropping solely. The effect was stronger with MeSA than with EBF on the control of *Rhopalosiphum padi*, and hoverflies and lacewings were twice more numerous in MeSA. These results show that combining wheat-pea intercropping with the release of EBF or MeSA can significantly reduce aphid density and attract their natural enemies and that this effect is strengthen with MeSA when compared to EBF.

Key words: Methyl salicylate ·*E*-β-farnesene ·Integrated pest management ·push-pull strategy ·semiochemical

1 Introduction

Aphids (Hemiptera: Aphididae) are the most dominant and destructive insect pests in wheat (*Triticum aestivum* L.) production regions in China (Cai et al. 2004), the two main species being *Sitobion avenae* (Fabricius) and *Rhopalosiphum padi* (Linnaeus) (Ma et al. 2006; Wang et al. 2009; Zhao et al. 2009). Aphids cause severe damages to wheat by feeding on leaves and developing ears, as well as by transmitting the barley yellow dwarf virus (Fereres et al. 1989). Lopes et al. (2016) reported that, in most of cases, aphids are reduced in wheat-based intercropping systems, compared to pure-stand crops. Hence, intercropping could be a promising practice in order to control aphids without chemical pesticides, which are harmful to health and the environment (Grung et al. 2015; Kim et al. 2017). Intercropping is defined as the cultivation of at least two plant species simultaneously in the same field, without necessarily being sown and/or harvested at the same time (Lithourgidis et al. 2011). It has been practiced in China for more than a thousand years and the benefits of mixing crops are being rediscovered in the light of the sustainability challenges agriculture faces (Knörzer et al. 2009). Among crops to be associated with wheat, pea (*Pisum sativum* Linn.) – as a legume – presents the interest of fixing atmospheric nitrogen and transferring it to the associated cereal plants, complementing or supplementing fertilizers (Bedoussac and Justes 2010; (Bedoussac and Justes, 2010; Hauggaard-Nielsen et al., 2008). Previous studies showed that the maintenance of pea cover between rows of wheat crop reduces populations of the wheat aphid *S. avenae* compared to pure-stand wheat (Zhou et al. 2009a, 2009b).

In addition to intercropping, the strategic deployment of semiochemicals (i.e. informative molecules used in insect-insect or plant-insect interactions) has been widely considered within Integrated Pest Management (IPM) programs (Heuskin et al., 2012a; Mensah et al., 2014; Nakashima et al., 2016; Rodríguez and Niemeyer, 2005; Sarles et al., 2015). Laboratory and field studies have demonstrated that releasing semiochemicals has the potential to simultaneously repel pests and attract natural enemies (i.e. 'push-pull' plant protection strategy) (Ninkovic et al. 2003; Zhou et al. 2016). Among other semiochemicals, methyl salicylate (MeSA) is a herbivore-induced plant volatile that is repellent to *R. padi* and other cereal aphids (Glinwood and Pettersson, 2000; Ninkovic et al., 2003). It is moreover attractive to aphid predators such as ladybeetles (Coleoptera: Coccinellidae; e.g. *Coccinella septempunctata* Linnaeus) (Saona et al., 2011; Zhu and Park, 2005), lacewings (Neuroptera: Chrysopidae; e.g. *Chrysopa nigricornis* Burmeister) (James, 2003a), hoverflies (Diptera: Syrphidae) (Mallinger et al., 2011), and aphid parasitoid wasps (Hymenoptera: Braconidae, Aphelinidae) (Gordon et al., 2013; Martini et al., 2014). Additionally, the aphid alarm pheromone – the major component being *E*-β-farnesene (EBF) (Francis et al., 2005) – acts as a repellent for plant herbivores and attracts predatory ladybeetles (e.g. *Harmonia axyridis*) (Francis et al., 2004; Verheggen et al., 2007), hoverflies (Verheggen et al. 2008a), lacewings (Boo et al., 1998), and parasitoids (Foster et al., 2005). To assess the effectiveness of different types of semiochemical in repelling pests and attracting their natural enemies, field experiments under production conditions are needed (Daems et al., 2016).

In their review, Lopes et al. (2016) highlighted that intercropping alone may not enhance pest natural enemies. Conversely, the use of semiochemicals in pure-stands may not be consistently successful and may even negatively influence natural enemies in low pest density situations (Wang et al., 2011b). Hence, combining semiochemicals with intercropping may bridge these problems. A previous experiment conducted in Belgium showed promising results toward the reduction of aphids and the increase of their natural enemies when wheat-pea intercropping was combined with the release of semiochemicals, compared to intercropping solely (Xu et al. 2017). The present study aims at evaluating this tactic in the context of China, by (i) determining if combining wheat-pea strip intercropping with the release of EBF or MeSA can better repel aphids and simultaneously attract their natural enemies than intercropping alone and (ii) evaluating the comparative efficacy of two types of semiochemicals (i.e. EBF and MeSA).

2 Materials and methods

2.1 Field layout

This study was conducted in fields of the Xinxiang experimental station of the Institute of Plant Protection, Chinese Academy of Agricultural Science, Henan Province of China (34°55′N, 114°15′E) in 2016. Three treatments, repeated three times, were tested: (1) wheat-pea strip intercropping (Control), (2) wheat-pea strip intercropping with EBF release formulated in oil (EBF), and (3) wheat-pea strip intercropping with MeSA release (MeSA). Repeated plots measured 80 m^2 (10 m x 8 m) and were placed in a completely randomized design within the field (Fig. 14).

Each plot was composed of three strips of winter wheat (variety 'Jimai 22', 225 kg seeds/ha) and two strips of winter pea (variety 'Zhongwan 4', 150 kg seeds/ha), each strip being 2 m wide. The two varieties are currently used commercially in Huang-Huai-Hai plain, China. Wheat and pea were separately sown on 20 October 2015 and 15 February 2016, respectively. Wheat and pea were harvested in June. All plots were irrigated during the growing season as standard agronomic practices used in northern China. The field was surrounded by strips of wheat (same variety) in order to limit the interactions with the surrounding fields. No pesticides (except fungicide) were used in the experimental area.

2.2 *E*-β-farnesene and methyl salicylate dispensers

EBF was provided by Prof. Frédéric Francis (Gembloux Agro-Bio Tech, University of Liège, Belgium), and was formulated in paraffin oil at a concentration of 10 mg/mL, MeSA (purity 99%) was purchased from Sinopharm Chemical Reagent Co., Ltd in China. For the experiment, 100 μL of EBF oil (10 mg/mL) for EBF treatment, and 400 μL of pure MeSA for MeSA treatment, was placed in a 1 cm-diameter rubber septum that was fixed to a trap stake in the middle of each plot. All release devices were placed under a plastic roof (35 × 35 cm) to protect them from the rain and they were changed every seven days.

According to previous studies, around 100 μg of EBF is released from 100 μL EBF oil over seven days in laboratory-controlled conditions (20 ℃, 65% relative humidity, airflow 0.5 L/min) (Heuskin et al., 2012b; Zhou et al., 2016). This information was used to calculate similar EBF daily release rates for field experiments. According to James (2003a), about 210 mg of MeSA was needed over seven days to obtain significant results, which would translate to about 468 mg MeSA released in our experiment. The first application of semiochemicals was on 21 March 2016.

2.3 Monitoring of aphids and beneficials

Aphids (all instars), their predators (i.e. larvae of ladybeetles, hoverflies and lacewings), and mummified aphids (mummies) were counted on pea plants and wheat tillers every seven days from 21 March 2016 to 28 May 2016 (9 weeks). Ten pea plants and 10 wheat tillers were randomly selected for counting insects at four different locations in each plot (totally 40 pea plants and 40 wheat tillers in each plot). Adults of ladybeetles, hoverflies, lacewings and alate aphids were collected using yellow pan traps (Flora®, 27 cm diameter and 10 cm depth). Traps were attached to fiberglass stakes, positioned at 10 cm higher than wheat, and filled with water and few drops of detergent to reduce water surface tension. A single trap was placed in the middle of each plot. Traps were emptied and refilled weekly during the same period. Trap contents were decanted through a 0.5 mm mesh sieve, and collected insects were transferred to plastic vials containing 75 % ethanol. Aphid predators and alate aphids trapped were identified in the laboratory to species level, using specific identification keys: : Taylor (1981) for aphids, Ren et al. (2009) for ladybeetles, He and Li (1992); Li (1988); van Veen (2010) for hoverflies, and Yang (1974) for lacewings. The number of individuals of each species was recorded.

2.4 Statistical analysis

One-way analyses of variance (ANOVA) were used to assess the effect of treatments (i.e. Control, EBF and MeSA) on the mean number of aphids (i.e. *S. avenae*, *R. padi*, pea aphids observed, *R. padi* winged aphids trapped), their natural enemies (trapped adults of ladybeetles, lacewings, hoverflies) and mummies. Mean abundances were compared between treatments by using Duncan multiple range tests. Insect abundances were $\log_{10}$ (x + 1)-transformed prior to analyses to achieve normal distributions. The analyses were performed by using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3 Results

3.1 Diversity and development trends of aphids and their natural enemies

Totally two species of aphid was observed on pea plants (*A. pisum*, *R. padi*) and four on wheat tillers (*S. avenae* and *R. padi, Metopolopium dirhodum* Walker, *Schizaphis graminum* Rondani) (Table 10). Pea aphids, *S. avenae* and *R. padi* observed exhibited a similar development trend in the three treatments (i.e. Control, EBF, MeSA). On wheat tillers, *S. avenae* and *R. padi* reached their abundance peak on 2 May and 9 May, respectively. Pea aphids reached their abundance peak about two weeks after *S. avenae* (Fig. 15). According to the identify result of trapped, species richness was highest for hoverflies (four species) and ladybeetles (four species), followed by lacewings (two species) (Table 10). The development trends of aphid natural enemies trapped were also not much affected by the release of EBF and MeSA, compared to the Control. They all reached their abundance peak on 21 May. The only exception concerned hoverfly adults, which abundance peak was advanced of about one week in MeSA treatment, compared to Control and EBF plots (Fig. 16). The peak of parasitized aphids (mummies) counted was observed on 15 May on wheat tillers, i.e. about one to two weeks after the peak of *S. avenae* and *R. padi* (Fig. 15). Finally, predatory larvae observed on both wheat and pea, as well as mummified aphids on pea, were very few abundant, which did not allow performing any further statistical analysis.

3.2 Effects of treatments on aphids and their natural enemies

The treatments significantly affected the abundance of pea aphids (F-value = 14.73; df = 2; P-value = 0.001), *S. avenae* (F-value = 11.33; df = 2; P-value = 0.003) and *R. padi* (F-value = 51.36; df = 2; P-value < 0.001) observed on plants. Duncan multiple range tests show that pea aphids, *S.avenae* and *R. padi* were significantly more abundant in the Control plots than in EBF and MeSA treatments (Table 11). *R. padi* were also less abundant in plots where MeSA was released than in all other treatments (Table 11). Additionally, the density of mummies was significantly affected by the treatments (F-value = 15.91; df =2; P-value = 0.004), being significantly less abundant on wheat tillers of the control plots than in EBF and MeSA treatments (Table 11).

In the traps, the treatments significantly affected the abundance of *R. padi* winged aphids (F-value = 19.88; df = 2; P-value = 0.002). Too few of the other four winged aphids (table 10) were collected in traps to permit statistical analysis. Treatments also affected the abundance of ladybeetle (F-value = 13.41; df = 2; P-value = 0.006), lacewing (F-value = 43.28; df = 2; P-value = 0.003) and hoverfly (F-value = 9.80; df = 2; P-value = 0.013) adults trapped. Duncan multiple range tests show that ladybeetles and lacewings were significantly less abundant in the Control plots than in EBF and MeSA treatments (Table 12). Moreover, lacewings were significantly more abundant in plots where MeSA was released, compared to those with EBF (Table 12). As for hoverflies, no differences were observed between EBF and Control treatments, but they were significantly more abundant in MeSA plots than in all other treatments (Table 12). More generally, aphid natural enemies were about two times more trapped in MeSA treatment than in the Control (Table 12).

4 Discussion

Releasing EBF or MeSA allows significantly reducing aphid density and attracting their natural enemies in intercropping systems in China. The beneficial effect of aphid reduction may be due to two factors. First, EBF and MeSA may have repelled aphids, and/or induced the development of wings, an effect that would accelerate aphid dispersal (Hatano et al., 2010; Kunert et al., 2005; Ninkovic et al., 2003; Thieme and Dixon, 2015). Second, the enhanced natural enemies may have preyed on and parasitized aphids, reducing their populations. As for natural enemies, ladybeetles and lacewings, which were the most abundant aphid natural enemies trapped, were positively attracted by both semiochemicals, confirming previous studies (Cui et al. 2012; Francis et al. 2004; James 2003, 2006; Zhu and Park 2005). As for the effect of EBF on lacewings, few experiments has been conducted in field conditions to our knowledge. Our present observations in fields are nevertheless not consistent with previous laboratory experiments using Y-tube olfactometer on the Asian lacewing *Chrysopa cognata* (Boo et al. 1998) and *Chrysopa pallens* (Li et al. 2017). However, previous electroantennogram results show that the antennae of *Chrysoperla carnea* are highly sensitive to EBF (Zhu et al. 1999), supporting the increased abundance of lacewings observed in EBF treatment compared to Control. Concerning hoverflies, they were not affected by EBF compared to Control, which contradicts previous findings reporting that EBF is an important olfactory cue for aphid location by hoverflies (Verheggen et al. 2008). However, hoverflies were positively affected by MeSA, which is consistent with (James, 2003b)). Finally, even though parasitoids were not identified from traps, the number of mummified aphids on wheat was higher in treatments with MeSA or EBF compared to Control, suggesting that releasing such semiochemicals in fields can enhance the host-finding ability of aphid parasitoids and leading to an improved pest control.

The present experiment also reveals that MeSA attracted twice more hoverflies and lacewings (and to a lesser extent ladybeetles) than EBF. This may explain the better control on *R. padi* in MeSA compared to EBF plots. To our knowledge, few studies previously compared the release of these two semiochemicals in wheat-pea intercropping systems toward biological control of aphids. Xu et al. (2017) showed in Belgium that ladybeetles were significantly more abundant in treatment with EBF in oil than with MeSA, while no significant differences were reported for lacewings and mummies, and hoverflies were increased in only one over the two years. This previous study also reported that pea aphids were about ten times more abundant than wheat aphids, while the contrary was observed here. These different results recall that insect dynamic may vary from a location to another, highlighting the needs to test tactics of biological control in various contexts. Nevertheless, in both studies, the release of the two semiochemicals led to a reduced abundance of aphids on both pea plants and wheat tillers, confirming their interest for IPM strategies.

Despite that this study was conducted over one growing season, the results show that releasing semiochemicals in intercropping systems allow reducing aphids and increasing their natural enemies. These results were stronger when MeSA was released, compared to EBF. Wheat-pea intercropping was previously shown to enhance 'associational resistance' to aphids (Ndzana et al., 2014b) and the addition of semiochemical releases can improve crop protection further by enhancing natural enemies while simultaneously repelling aphids.

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Figures and Tables:

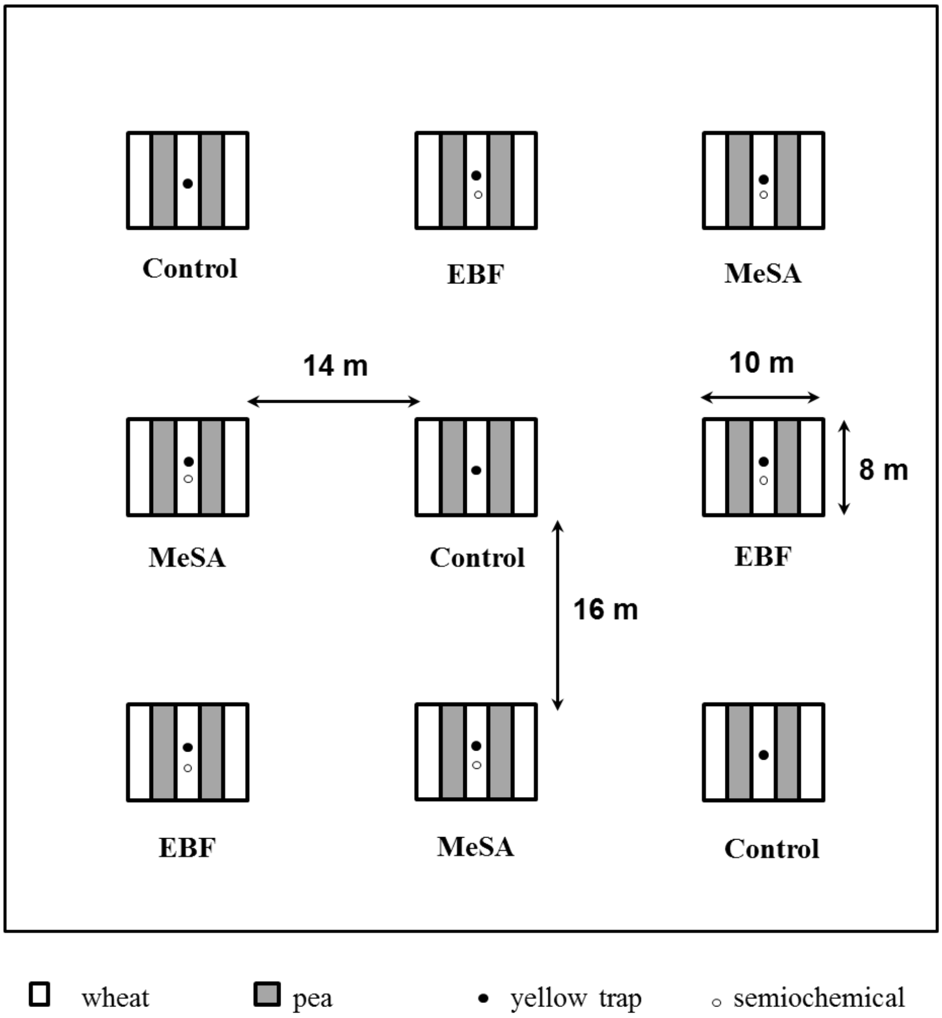

Figure 14 Experimental design (wheat-pea intercropping (control), wheat-pea intercropping with EBF release using oil (EBF), wheat-pea intercropping with MeSA release (MeSA))

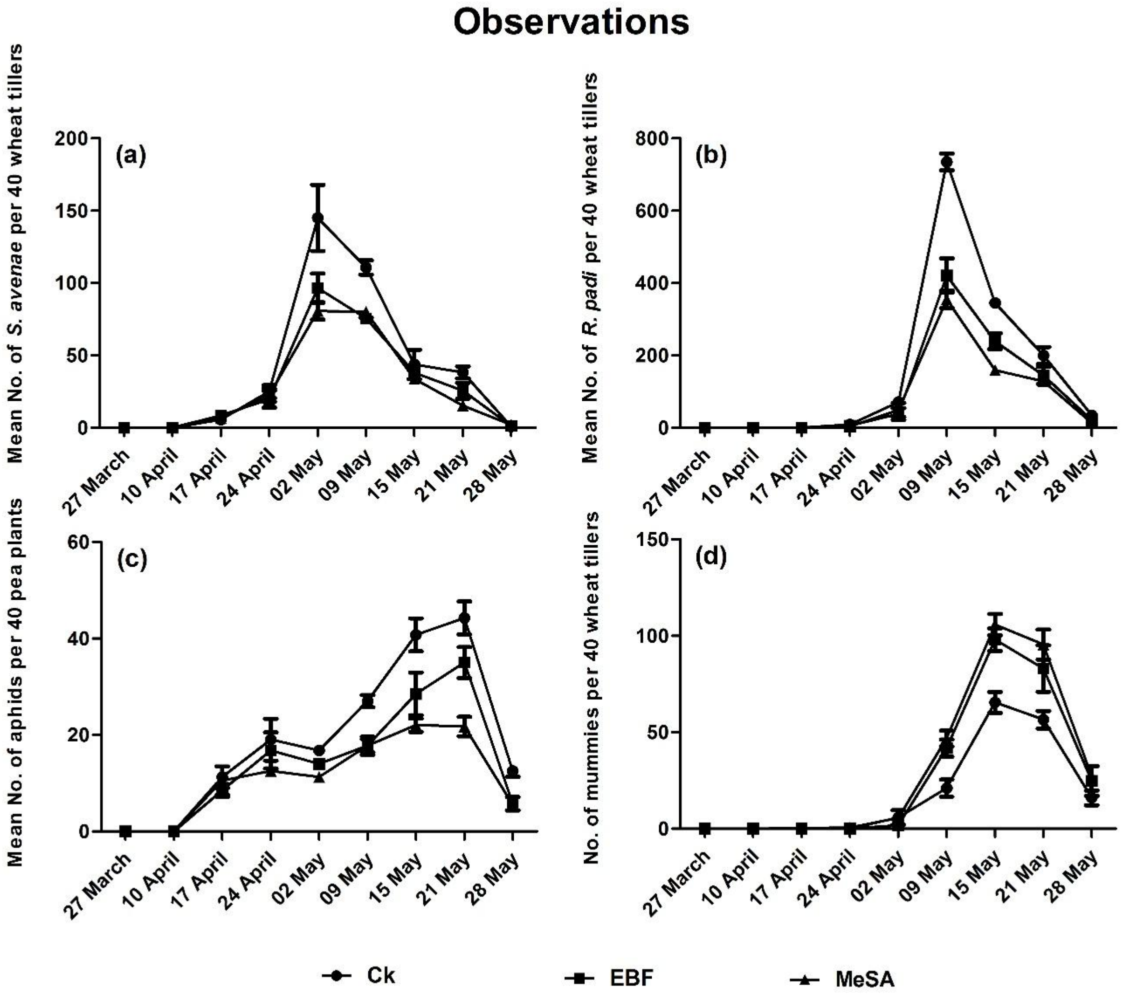

Figure 15 Mean numbers (and standard error) of aphids and mummies on the pea and wheat per 40 plants/tillers. a) *S.avenae* on the wheat, b) *R. padi* on the wheat, c) Aphis on the pea, d) Mummies on the wheat.

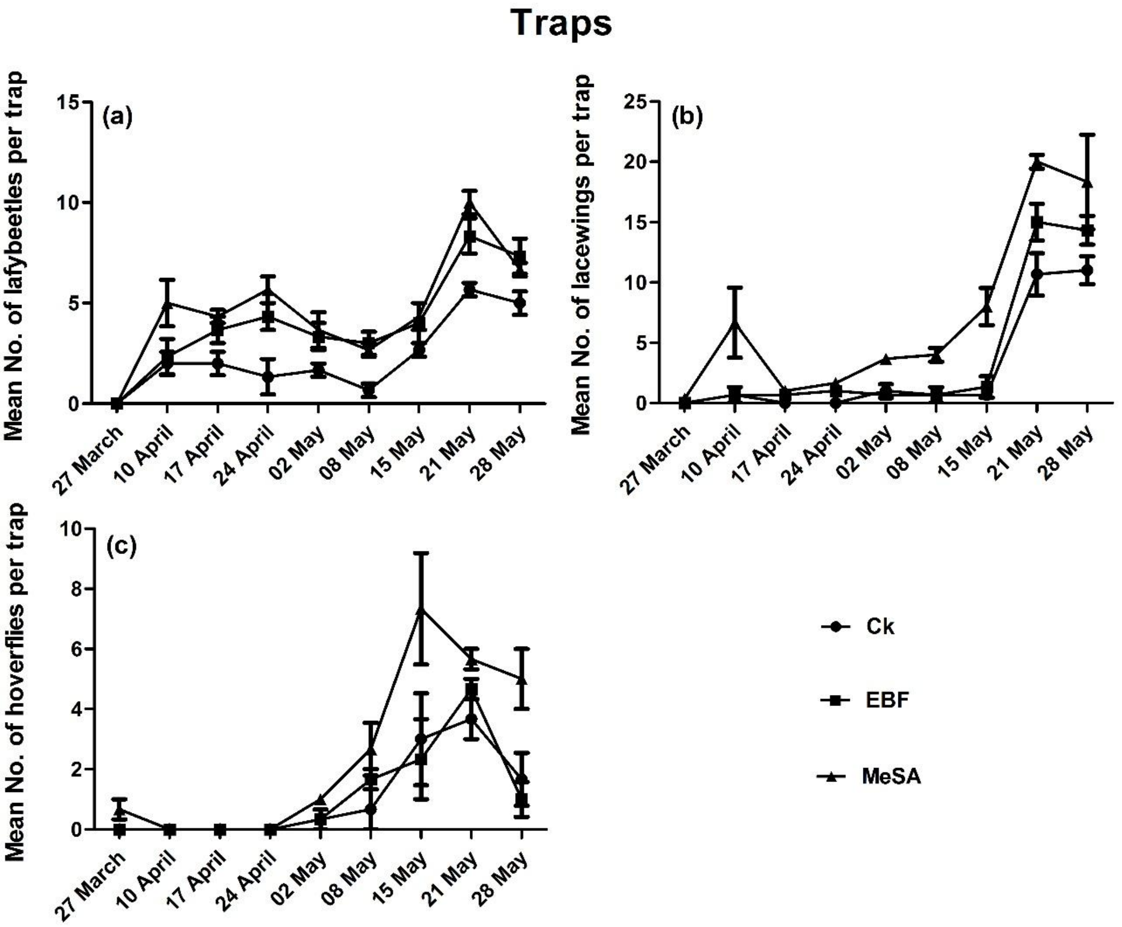

Figure 16 Mean numbers (and standard error) of natural enemies recorded in different treatments throughout 2016 growing season. a) Ladybeetles, b) Lacewings, c) Hoverflies.

Table 10 Diversity of aphids and their trapped natural enemies

Order: Family	**Species**
Aphids (Hemiptera: Aphididae)	*Sitobion avenae* (Fabricius)
	Rhopalosiphum padi (Linnaeus)
	Metopolophum dirhodum (Walker)
	Schizaphis graminum (Rondani)
	Acyrthosiphon pisum (Harris)
Ladybeetles (Coleoptera: Coccinellidae)	*Harmonia axyridis* (Pallas)
	Coccinella septempunctata (Linnaeus)
	Propylaea japonica (Thunberg)
	Adonia variegate (Goeze)
Hoverflies (Diptera: Syrphidae)	*Episyrphus balteata* (De Geer)

	Metasyrphus corollae (Fabricius)
	Sphaerophoria scripta (Linnaeus)
	Scaeva pyrastri (Linnaeus)
Lacewings (Neuroptera: Chrysopidae)	*Chrysopa sinica* (Tjeder) *Chrysopa septempunctata* (Wesmael)

Table 11 Mean number (and standard error) of aphids on the pea and wheat recorded in different treatments throughout 2016 growing season. (Letters indicate significant differences based on Duncan multiple range test on SAS 9.4 ($p < 0.05$))

	Observations on pea	**Observations on wheat**		
Treatment	**Aphids**	***S. avenae***	***R. padi***	**Mummies**
Control	171.00±12.50a	369.00±31.34a	1394.75±44.73a	173.00±9.54a
EBF	126.25±10.89b	265.00±19.95b	869.25±53.99b	247.00±22.50b
MeSA	101.25±3.64b	240.75±3.04b	707.75±30.57c	275.67±6.17b

Table 12 Mean numbers (and standard error) of natural enemies in the traps in different treatments throughout 2016 growing season. (Letters indicate significant differences based on Duncan multiple range test on SAS 9.4 ($p < 0.05$))

Treatment	**Winged aphids**	**Natural enemies**		
	R. padi	**Ladybeetles**	**Lacewings**	**Hoverflies**
Control	284.33±6.34a	21.00±2.52a	24.67±1.76a	9.33±1.76a
EBF	238.33±9.52b	36.33±4.91b	34.33±2.73b	10.00±1.53a
MeSA	222.67±4.91b	42.33±1.45b	63.67±4.10c	22.33±1.67b

Chapter VI Gerneral discussions and conclusions

Wheat is an essential food crop in northern China and in Europe. Aphids is an important pest that attacks wheat throughout its growth stages in north China (Wang et al., 2009; Zhou et al., 2009). The aphids feed on phloem sap, causes substantial losses of cereal yield by sucking the juice and transmitting viruses. Application of chemical pesticides is still the main method to control aphids; however, chemical control causes negative impacts on agroecosystems and can lead to insect resistance to pesticides (Wei and Huang, 1988). Thus searching for alternative methods to control this aphid is of great significance.

Fifty original research papers were obtained from a systematic search of the peer-reviewed literature. Results from a vote-counting analysis indicated that, in the majority of studies, pest abundance was significantly reduced in intercropping systems compared with pure stands. However, the occurrence of their natural enemies as well as predation and parasitism rates were not significantly increased. The country where the studies took place, the type of intercropping, and the crop that was studied in the association had significant effects on these results.Wheat-based intercropping systems almost systematically have a positive effect on pest control. In fact, the number of responses reporting a decrease of their populations was significantly higher than those showing the opposite. This finding is consistent with most studies addressing the effect of plant diversity on herbivores (Andow, 1991; Letourneau et al., 2011b). This study shows that wheat-based intercropping systems allow reducing pest occurrence on crops, while natural enemies are not favoured in such systems when compared to pure stands. Because predators and parasitoids are not significantly favoured in intercropping systems, these latter could be combined with other practices known to efficiently support natural enemies within fields. For instance, some volatiles known to attract natural enemies can be released in fields. Wang et al. (2011) showed that the abundance of ladybeetles and parasitism rate were higher when methyl salicylate was released in wheat-oilseed rape intercropping fields, compared to each treatment applied separately. The conservation of natural enemies and their attraction in intercropping fields could be a way to improve the biological control of pests.

A brief research was conducted in order to have an overview of the intercropping on the spread of aphids, to assess the potential impact of intercropping systems attracting natural enemies on the virus transmission. Dispersal of viruses is intimately tied to their vectors. Aphids are known to invest in costly antipredator behavior when perceiving cues of predators. We studied aphid antipredator behavior in intercropping with wheat -broad bean as a model. *R. padi* is an important vector of the barley yellow dwarf virus. The effects of two natural aphid enemies, adult and larvae of the seven-spot ladybeetle, *Coccinella septempunctata* Linneaus (Coleoptera:

Coccinellidae), on *R. padi* dispersion was studied under laboratory conditions. Trays composed of 7 × 8 lines of plants were considered. In intercropping, one line of broad-bean succeeded one line of wheat. Aphids and predators were introduced on wheat tillers in the middle of the system (source line) and aphids were counted on every plants after two and 24 hours. Results show that the total number of aphids was higher in intercropping than monoculture in treatments without ladybeetles, while the contrary was observed in the presence of ladybeetle larvae, it shows that this dispersion was limited in intercropping systems. Nevertheless, a reduced abundance of aphids on the source line in the presence of ladybeetle larvae was observed, which may be due to the feeding behavior of the predators, or the aphids dropping from plants (Belliure et al., 2011). In intercropping systems, non host plants can represent chemical and physical barriers limiting the ability aphids to find their host plants after being dropped from wheat (Lopes et al., 2015b). Predator size and foraging speed have been noticed as factors used by aphids to assess predation risks, also, the consumption rate of *C. septempunctata* larvae was much higher than the adult one (Brodsky and Barlow, 1986). Understanding how intercropping affects vector populations and behavior spread would participate in assessing how such a practice may affect pathogen spread. Several studies evaluated the impact of intercropping on disease spread for vector-borne viruses. Fargette and Fauquet (1988) suggested that mixed cropping including cassava (*Manihot esculenta* Crantz) may allow decreasing whitefly (Hemiptera: Aleyrodidae) vector populations, hence the spread of cassava mosaic disease. Moreover, Fondong et al. (2002) observed that cassava intercropped with maize or cowpea (*Vigna unguiculata* L.) allows decreasing adult whitefly populations on cassava by 50% and cassava mosaic disease incidence by 20%. Therefore, we can hypothesize that intercropping can reduce the transmission of BYDV by *R. padi*. Nevertheless, such a hypothesis remains to be tested. These results might be explained by the non-host plant chemical cues and the physical barrier that was broad-bean plants confusing *R. padi* when searching for their host plants after being dropped from wheat by predators (i.e. associational resistance). This study shows that intercropping can reduce the dispersal of aphids in the presence of predators, *in fine* potentially limiting virus dispersal.

To solve how to increase the number of natural enemies of intercropping in Belgium and China. In Belgium, a two-year (2015, 2016) setup involving wheat-pea strip intercropping combined with the release of E-β-farnesene (EBF) or methyl salicylate (MeSA) was tested as a push–pull strategy to simultaneously repell aphids and attract beneficials. Two types of slow-release formulation (i.e., oil and alginate beads) containing EBF or MeSA were deployed with intercropping. The abundance of aphids was significantly decreased, hoverfly larvae and mummified aphids increased on both pea plants and wheat tillers by the release of oil-formulated EBF and MeSA. The proportion parasitism of aphids-parasitism rate was also increased by treating both crops in both years. Releasing EBF through oil rather than alginate beads proved significantly better for attracting natural enemies and reducing aphids.

The semiochemicals EBF and MeSA are repellent to aphids, but are also known to induce the development of wings, an effect that would accelerate aphid dispersal (Hatano et al. 2010; Kunert et al. 2005; Ninkovic et al. 2003; Thieme and Dixon 2015). Secondly, the natural enemies attracted by these compounds probably preyed on and parasitized the aphids, reducing their populations. Hoverflies, which were the most abundant aphid natural enemies, were positively attracted by the semiochemicals and were negatively correlated with aphid abundance. Previously, Verheggen et al. (2008) showed that EBF is an important olfactory cue for aphid location by hoverflies and that it induces oviposition, whereas Francis et al. (2005) showed EBF to be an important olfactory cue for aphid location by hoverfly larvae. As for , the present results for MeSA are consistent with those of James (2003) who observed it to attract hoverfly adults in the field. EBF was also found to be attractive to adult lady beetles, which is consistent with previous studies on several species (Cui et al. 2012; Francis et al. 2004; Leroy et al. 2012). Nevertheless, the lack of an effect of MeSA on lady beetle recruitment contradicts the results of previous field studies (Rodriguez-Saona et al. 2011; Zhu and Park 2005). However, MeSA did attract lacewings, as found by James (2003a, 2006), who studied several lacewing species including *Chrysopa nigricornis* and *Chrysopa oculata* (Neuroptera: Chrysopidae).

The release of EBF from oil rather than alginate beads was significantly more effective in attracting natural enemies and reducing aphid densities. Some of this difference could be explained by differential sensitivity of release formulations to climatic conditions. Whereas our results support oil to be more effective in such regions, further studies might test alginate beads in controlled environments such as greenhouses.

In China, one year (2016) setup involving wheat-pea strip intercropping combined with the release of EBF or MeSA was tested as a push-pull strategy to simultaneously repell aphids and attract beneficials. The total number of aphids throughout the growing season was significantly decreased in treatments with releases of semiochemicals compared to intercropping solely. The effect was stronger with MeSA than with EBF on the control of *R. padi*, and hoverflies and lacewings were twice more numerous in MeSA. These results show that combining wheat-pea intercropping with the release of EBF or MeSA can significantly reduce aphid density and attract their natural enemies and that this effect is strengthen with MeSA when compared to

EBF. Releasing EBF or MeSA allows significantly reducing aphid density and attracting their natural enemies in intercropping systems in China. The beneficial effect of aphid reduction may be due to two factors. First, EBF and MeSA may have repelled aphids, and/or induced the development of wings, an effect that would accelerate aphid dispersal (Hatano et al., 2010; Kunert et al., 2005; Ninkovic et al., 2003; Thieme and Dixon, 2015). Second, the enhanced natural enemies may have preyed on and parasitized aphids, reducing their populations.

The present experiment also reveals that MeSA attracted twice more hoverflies and lacewings (and to a lesser extent ladybeetles) than EBF. This may explain the better control on *R. padi* in MeSA compared to EBF plots. To our knowledge, few studies previously compared the release of these two semiochemicals in wheat-pea intercropping systems toward biological control of aphids. Xu et al. (2017) showed in Belgium that ladybeetles were significantly more abundant in treatment with EBF in oil than with MeSA, while no significant differences were reported for lacewings and mummies, and hoverflies were increased in only one over the two years.

Nevertheless, in both studies, the release of the two semiochemicals led to a reduced abundance of aphids on both pea plants and wheat tillers, confirming their interest for IPM strategies. In agricultural systems, the goal is to maximize output from the whole system while minimizing cost. Predators and parasitoids can make valuable contributions to biological control in IPM and many are commercially available for inundative release. The push-pull system effectively addresses the constraints to cereal production faced by the farmers and is an appropriate system because it uses locally available companion plants rather than expensive imported inputs. It is, thus, a novel IPM approach that was developed with full participation of the target farmers.

In future, in the field, we will continue test two types of slow-release formulation (i.e., oil and alginate beads) of MeSA in China, to test different climatic conditions on the MeSA release efficiency, eventually affecting on the pest control efficiency. In addition, we will assess the reducing pesticide efficiency of combining intercropping with semiochemical strages, to guide farmers in field applications. Zhang et al. (2017) demonstrate that the EBF is detected in the pea aphid by odorant receptor ApisOR5 with the cooperation of odorantbinding proteins ApisOBP3 and ApisOBP7, in the labratory, we try to analyze the olfactory recognition mechanism of EBF with ladybeetles, try to find the relevant odorant receptors and odorant-binding proteins.

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Chapter VII List of scientific productions

Publications included in the thesis

1. **Qingxuan Xu**, Séverin Hatt, Thomas Lopes, Yong Zhang, Bernard Bodson, Julian Chen, Frédéric Francis. (2017) A push-pull strategy to control aphids combines intercropping with semiochemical releases. Journal of Pest Science DOI: 10.1007/s10340-017-0888-2.
2. **Qingxuan Xu**, Séverin Hatt, Thomas Lopes, Julian Chen, Frédéric Francis. (2017) Antipredator response of aphids to ladybeetles: effect of intercropping on aphid dispersal. Comm. Appl. Biol. Sci, Ghent University (the 69th ISCP).
3. Lopes T. & Hatt S., **Qingxuan Xu**, Chen JL., Francis F. Wheat (*Triticum aestivum*, L.)-based intercropping systems for biological pest control: a review. Pest Management Science, 2016, 72: 2193-2202.
4. **Qingxuan Xu**, Séverin Hatt, Zongli Han, Frederic Francis, Julian Chen. (2017) Combining E-β-farnesene and methyl salicylate release with wheat-pea intercropping enhances biological control of aphids in China. BioControl (under review).

Other publications not related to the subject

1. **Qingxuan Xu**, Jia Fan, Zongli Han, Yong Zhang, Frédéric Francis*, Julian Chen* (2017) Effect of protein elicitor PeaT-1 on the control of wheat aphids in the laboratory and in the field. Journal of Integrative Agriculture (under review).
2. Xue W, Fan J, Zhang Y, **Qingxuan Xu**, Han Z, Sun J, et al. (2016) Identification and Expression Analysis of Candidate Odorant-Binding Protein and Chemosensory Protein Genes by Antennal Transcriptome of *Sitobion avenae*. PLoS ONE, 2016, 11(8): e0161839.
3. Séverin Hatt, Thomas Lopes, **Qingxuan Xu**, et al. (2016) Controlling aphids on wheat by sowing wildflower strips within field: it's possible! NSABS 2017.
4. Séverin Hatt, **Qingxuan Xu**, Frédéric Francis, Julian Chen (2017) Does wheat - oilseed rape intercropping combined with the slow release of ladybeetle sex pheromone allow reducing aphids and increasing their natural enemies in China? Journal of Asia-Pacific Entomology (under review).

Communications:

1. **Qingxuan Xu**, Jia fan, Frederic Francis, Julian Chen. Effect of three wheat intercropping model on the population dynam ics of *Sitobion avenae* and its main natural enemies. Research presentations & summary, China Plant Protection Society Annual Conference Proceeding 2014.
2. **Qingxuan Xu**, Thomas Lopes, Séverin Hatt, Frédéric Francis, Julian Chen. Effect of two types of semiochemical on population development of aphids in wheat-pea intercropping in Belgium. Research presentations & summary, China Plant Protection Society Annual Conference Proceeding 2016.
3. Séverin Hatt, **Qingxuan Xu**, Zongli Han, Frédéric Francis, Julian Chen. Combining intercropping with the release of ladybeetle's sexual pheromone for controlling aphids and favor their natural enemies on wheat. Research presentations & summary, China Plant Protection Society Annual Conference Proceeding 2016.
4. **Qingxuan Xu**, Jia fan, Frederic Francis, Julian Chen et al. Ecological control effects on wheat aphids of protein elicitors-PeaT1. Newsletter of the Entomological Society of Beijing, No.28, 16 April 2016.
5. Séverin Hatt, & Thomas Lopes, **Qingxuan Xu**, Yong Liu, Frédéric Francis, Julian Chen. Effect of wheat-based intercropping systems on pests and natural enemies: a review with a special focus on China. Newsletter of the Entomological Society of Beijing, No.28, 16 April 2016.
6. **Qingxuan Xu**, Séverin Hatt, Thomas Lopes, Yong Zhang, Bernard Bodson, Julian Chen, Frédéric Francis. A push-pull strategy to control aphids combines intercropping with semiochemical releases in Belgium and China. 1st International Conference on Integrated Pest Management, Changsha, China, 20 July 2017.

Posters

1. **Qingxuan Xu**, Séverin Hatt, Thomas Lopes, Julian Chen, Frédéric Francis. (2017) Antipredator response of aphids to ladybeetles: effect of intercropping on aphid dispersal. The 69th ISCP, 23 May 2017.