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Use of intercropping and infochemical releasers to control aphids in wheat

Haibo ZHOU

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Abstract: 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 decrease yield, and they are varieties virues vector. In this context, the main objective of this thesis was to promote the intercropping and infochemical releasers as Integrated Pest Management (IPM) agents by developing alternative strategies for aphid control in wheat crop. Two different approaches have been adopted here with success: (1) the potential use of semiochemical releaser for aphid control, and (2) the use of flowering plant as a habitat management tool to enhance biological control of aphids.

Firstly, field experiments were performed in wheat exploiting semiochemical from plant essential oils affecting population density in cereal aphids and their natural enemies. Results showed that: (Z)-3-hexenol (Z3H) has shown the attractiveness to *Metopolophum dirhodum* (Walker) and *Sitobion avenae* (Fabricius), and (E)-β-farnesene (EBF) and garlic extraction (GE) repelling the two aphids. The high population of hoverflies and lacewing fly were found in EBF and GE treatments, respectively. The results promoted the "push-pull" strategy in aphid biological control that Z3H could be regard as the pull stimulus, and GE and EBF as the push stimulus .

Secondly, the laboratory test for beneficial effect of associating pea to wheat showed that the frequencies of searching and oviposition parameters of hoverfly were influenced by the selected combinations. In addition, the oviposition frequency of *Episyrphus balteatus* was improved when related to the presence of pea in wheat plants. Odors from combinations of wheat and pea had limited effect on the preference of *Harmonia axyridis.* Healthy wheat plants were preferred by *S. avenae* to empty control. Also, the presence of conspecific on wheat proposed plant did not provide any more attraction to *S. avenae* alate. The presence of *Acyrthosiphon pisum* infested pea induced a significant repellent effect on *S. avenae*.

Finally, based on the beneficial effect of associating pea to wheat, the field experiments of wheat-pea intercropping or mixing were performed in China and Belgium. We found that the high abundance of hoverflies, lacewing fly and ladybirds were found in wheat mixed with pea field, but low population of cereal aphids in diversified wheat field. The Land equivalent ratio, 1.121-1.187 for wheat-pea intercropping in 2008 and 1.114-1.174 for wheat-pea intercropping in 2009, showed that intercropping of wheat and pea has a potential to improve the utilization of plant growth resources as compared to sole crops.

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Chapter I: *GENERAL INTRODUCTION*

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 yield-sapping pathogens. Moreover, honeydew, the aphid excretory product, rich in sugars and amino acids, also provides an ideal environment for the development of saprophytic fungal organisms, which reduces transpiration and photosynthesis, affecting growth and development of the plant. Among aphid species, the rose grain aphid *Metopolophum dirhodum* (Walker), English grain aphid *Sitobion avenae* (Fabricius) and bird cherry-oat aphid *Rhopalosiphum padi* (Linnaeus) attack a range of small grains, causing economic damage and necessitating routine insecticide use.

Crop monocultures of genetically homogeneous encourages the evolution, multiplication and spread of newly adapted weed, pest insect and pathogen on massive and uniform crop. It has led to many well-known problems such as soil erosion, environmental contamination by fertiliser and pesticides, and disease, pest or weed resistance to pesticides. 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.

To reduce reliance on this pesticide use and associated economic, environmental, and health 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.

Chapter II: *THE OVERVIEW OF BIODIVERSITY CONSERVATION FOR THE PEST MANAGEMENT IN AGROECOSYSTEMS*

The overview of biodiversity conservation for the pest management in agroecosystems

Haibo Zhou[a,b], Julian Chen[b], Yong Liu[c], Claude Bragard[d], Eric Haubruge[a], Frédéric Francis[a]

[a]Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux, 5030, Belgium

[b]State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China

[c]College of Plant Protection, Shandong Agricultural University, Taian, 271018, PR China

[d]Applied Microbiology-Phytopathology, Earth and Life Institute, Université catholique de Louvain, Louvain-la-Neuve,1348, Belgium

Abstract: Biodiversity, longer term benefits for sustainability of the farming system, provides an ecologically based approach aimed at favouring natural enemies and enhancing biological control in agricultural systems. Numerous studies have evaluated the impact of plant diversification on pests and beneficial arthropods population dynamics in agricultural ecosystems and provided some evidence that habitat manipulation techniques (e.g. intercropping, undersown nonhost plants, vegetation borders) benefited pest control. In many instances, mechanisms accounting for herbivores and natural enemy responses to plant diversification are not thoroughly tested. The rapidly expanding literature on biodiversity is reviewed with attention to the ways in which agricultural biodiversity may be increased to favour pest management, the contributions of plant diversification, and mechanisms influencing arthropods response to plant diversification to this developing area of conservation biological control. Various potential options of habitat management and design that enhance functional biodiversity in crop fields are described and discussed. Quantitative data are needed to determine the level of change in plant quality brought about by companion planting that alters arthropod behavior.

Key words: Biodiversity, agroecosystem, pest insect, biocontrol.

1. Introduction

Currently, studies in integrated pest management emphasize biological interactions among insect pests, natural enemies, and plants, which have led to a recent renaissance in interest and research activities on cultural and biological controls in entomology. Modern agriculture implies the simplification of the structure of the environment over vast areas, replacing nature’s diversity with a small number of cultivated plants and domesticated animals. Commercial seed-bed preparation and mechanized planting replace natural methods of seed dispersal; chemical pesticides replace natural controls on populations of weeds, insects, and pathogens; and genetic manipulation replaces natural processes of plant evolution and selection (Altieri, 1999). It has led to many well-known problems such as soil erosion, environmental contamination by fertiliser and pesticides, and disease, pest or weed resistance to pesticides (Jackson and Piper, 1989, Bedoussac and Justes, 2011). Hence, there is consequently a need to develop new arable cropping systems for greater efficiency and resource conservation.

Recently, increasing attention has been paid to conservation practices that seek to increase the biodiversity in agroecosystems. Enhancing functional biodiversity in agroecosystems is a key ecological strategy to bring sustainability to production (Altieri, 1999). In Latin America farmers grow 70-90% of their beans with maize, potatoes and other crops. Maize is intercropped on 60% of the region’s maize-growing area (Francis, 1986). In a detailed, quantitative review, Andow (1991) found that although natural enemy densities tended to be greater in polycultures than in monocultures, only slightly more than half of the 287 herbivore species were consistently less abundant in polycultures. One reason for this inconsistent effects of enhanced vegetational biodiversity is that the effects of different types of plants on natural enemies can vary markedly (Colley and Luna, 2000). Despite such potential problems, there are many successful instances of biodiversity being used in agroecosystems to favour natural enemies, suppress pests and, in some cases at least, reduce crop damage. Perrin (1976) suggested that because polycultural cropping systems are so prevalent in many areas of the world, it behooves us to understand the ecology of arthropod response to polyculture in order to improve pest management in these systems. Whilst an understanding of the mechanisms by which biodiversity may favour pest management is important (Gurr et al., 2003).

Although agricultural land holds much of the world’s biodiversity (Pimentel et al., 1992), the relative contribution of each management type to conservation is little known (Tscharntke et al., 2005). The focus of this review is the application and mechanisms of biodiversity in agricultural systems to enhance pest management. We present a concise overview of the ways in which this may be achieved. Full recognition of such multi-function agricultural biodiversity can serve only to encourage appropriate societal incentive schemes and consequent adoption by farmers.

2. The biodiversity in agricultural ecosystems

Agricultural biodiversity is a fundamental feature of farming systems around the world (Thrupp, 2000). Agrobiodiversity therefore includes not only a wide variety of species and genetic resources, but also the many ways in which farmers can exploit biological diversity to produce and manage crops, land, water, insects and biota (Brookfield and Padoch, 1994). The concept also includes habitats and species outside farming systems that benefit agriculture and enhance ecosystem functions. One example is a source of host plants for natural enemies and predators of agricultural pests (Thrupp, 2000). The study of effect of biodiversity in agricultural ecosystems on herbivores and their natural enemies has focused on wheat, maize, cotton, vegetables and so on (Table 1).

Biodiversity refers to all species of plants, animals and micro-organisms existing and interacting within an ecosystem (Vandermeer and Perfecto, 1995). During the last decades, worldwide losses of biodiversity have occurred at an unprecedented scale and agricultural intensification has been a major driver of this global change (Tilman et al., 2001, Tscharntke et al., 2005). Most studies conclude that by mixing certain plant species with the primary host of a specialized herbivore gives a fairly consistent result: specialized herbivore species usually exhibit higher abundance in monocultures than in polycultures (Altieri, 1999). When a species grown as a sole crop is attacked by herbivorous pest, it is often found that the same species grown intercropped with other sorts of plant shows less abundance of pest (Trenbath, 1993). This is especially true where the attacking organism has a narrow host range (Andow, 1991).

3. The contributions and mechanisms of biodiversity

3.1 Contributions

Plants in diversification of the ecological system may sustain lower herbivore populations because herbivores have difficulty finding them, leave them more quickly, or have difficulty relocating them after leaving (Andow, 1991). Behavioral observation can demonstrate that an herbivore has difficulty finding its host, although this demonstration can be complicated. Elmstrom et al (1988) showed that polycultures reduced host-finding and increased host-leaving rates compared to monocultures. A three-year field experiment conducted by Tahvanainen and Root (1972) showed that adult *Phyllotreta cruciferae* were more abundant on collards grown in monocultures than that in stands in which collards had been interplanted with tomatoes and tobacco. Major insect pests and their natural enemies were sampled on cowpea in monocropping and cereal intercropping plots in southern and northern Nigeria. Populations of flower thrips, *Megalurothrips sjostedti* (Trybom), were reduced by 42% and predators, mostly *Orius spp*. (Anthocoridae), by 23% on cowpea in maize intercropping plots at Ofiki in the south, and infestation by pyralid pod borer, *Maruca testulalis* Geyer, was unaffected by cropping system (Matteson, 1982). Those studies suggest that the more diverse the agroecosystems and the longer this diversity remains undisturbed, the more internal links develop to promote greater insect stability.

Enhanced agricultural biodiversity is known to: (1) reduce pests and diseases (Altieri, 1999), (2) attract natural enemies (Trenbath, 1993, Östman et al., 2001), (3) favour weed control (Banik et al., 2006), (4) improve soil conservation (Gurr et al., 2003), (5) provide better lodging resistance (Anil et al., 1998), (6) improve stability of ecosystem (MacArthur, 1955, Pimentel, 1961), (7) increase yield and grain protein concentration (Bedoussac and Justes, 2011) and (8) regulate microclimate within agroecosystems (Brust et al., 1986, Altieri, 1999, Gurr et al., 2003) compared with simplified vegetation in farm and landscape scales (Fig.1) (Gurr et al., 2003). These effects may extend both spatially to adjacent crops and temporally to subsequent crops, so increasing the sustainability of the farming system.

Altieri (1999) have developed several types of diversified agroecosystems related to weeds, annual polyculture, complex perennial crop and adjacent vegetation. Overwhelming evidence suggests that diversified agroecosystems could support a lower herbivore load than simple cultures. One factor explaining this trend is that relatively more stable natural enemy populations can persist in polycultures due to the more continuous availability of food sources and micro habitats. The other possibility is that specialized herbivores are more likely to find and remain on pure crop stands that provide concentrated resources and monotonous physical conditions. Trenbath (1993) reviewed that the presence of associated plants in the intercrop can lead to attack escape in three ways. In one, the associates cause plants of the attacked component to be less good hosts; in the second, they interfere directly with activities of the attacker; and in the third, they change the environment in the intercrop so that natural enemies of the attacker are favoured.

The effect of biodiversity in agriculture should be varied across agroecosystems which differ in crop species. In addition, particular arthropod herbivores respond to polycultures differently depending on the number of host plants in the polycultures (Andow, 1991). For example, the cicadellid *Scaphytopius acutus* had higher population density on peach trees associated with a ground cover of red clover or mixed rosaceous weeds, which are favored host plants, but lower population density on peach trees associated with a ground cover of a nonhost grass compared to monocultures (McClure et al., 1982).

3.2mechanism

Several ecological factors or hypotheses have been offered to explain why insect communities in agroecosystems can be stabilized by constructing vegetational architectures that support natural enemies and/or directly inhibit pest attack. Efforts to disentangle the reasons for the reduced populations of herbivorous pest and associated lighter damage in biodiversity systems have provided a fascinating array of possible mechanisms mostly relatable to microenvironmental effects of the associated crop (Letourneau, 1990). Trenbath (1993) also summarized the mechanisms for pest seem to fall into the following three main categories: (1) indirect effects on the attacking pest through changes in the plants of the attacked component which affect their "quality" as host plants; (2) direct effects on the pest, how it colonises its hosts, grows and reproduces; and (3) a further set of indirect effects on the attacking pest, but here through the pest's own natural enemies, its predators or parasites, how they find or colonise the pest, how they grow and reproduce. Examples of the operation of these factors of all mechanisms below have been found in pest attack, but individual cases often involve more than one.

3.2.1 Olfactory

The diversity of olfactory stimuli emanating from polycultures might mask the olfactory cues used by monophagous herbivores to find their host plants or otherwise confuse or repel these herbivores (Andow, 1991). In a choice test between host plants with tomato or ragweed odors versus host plants alone, Tahvanainen & Root (1972) showed that *P. cruciferae* was more likely to move to host plants alone than host plants associated with nonhost odors. Strongly aromatic crops such as garlic and tomato can provide an olfactory camouflage against insects which masks their normal host-finding or feeding cues (Perrin and Phillips, 1978). Where one crop gives off an apparently repellant odour, an associated crop can be strongly protected from some species (Atsatt and O'Dowd, 1976). The presence of a lower storey of crop or weeds can similarly affect visual search (Altieri et al., 1990).

However, an herbivore with highly sensitive receptors will be able to respond to subtle quantitative differences in concentration gradients of host odors because it can detect the very low concentrations far from the host stand. Stanton (1983) proposed a simple model of host-plant finding by herbivores using long distance olfactory stimuli. Herbivores respond to their olfactory stimuli upon random encounter with a part of the odor plume in which odor concentration is greater than their receptor sensitivity, then host finding by herbivores with low olfactory sensitivity is unlikely to be affected by polycultures. These ideas have not yet been critically tested.

3.2.2 Visual

Plant architecture also plays a role in tritrophic interactions (Marquis and Whelan, 1996). Architectural traits of plant, which include stem or leaf dimensions, branching angles, surface complexity, and canopy spacing, may also "guide" enemy searching and influence either the time a predator spends on a plant or the overlap between predator and prey distributions (Ferran and Deconchat, 1992, Frazer and McGregor, 1994). For example, comparisons between aphid-free and aphid-infested plants suggest that differences in plant architecture modified prey accessibility rather than predator movement (Clark and Messina, 1998).

Biodiversity might also interfere with visual host finding cues. For instance, cabbage aphid, *Brevicoryne brassicae* colonization of brussels sprouts was less in polycultures than monocultures and was less when green burlap was placed between host plants than when brown burlap was so placed. The nonhost plants and the green burlap may have reduced the contrast between green plants and brown soil and made the host plants less attractive to colonizing aphids (Smith, 1976).

3.2.3 Host-plant quality

Host-plant quality can influence herbivore host finding because different quality plants can release different concentrations of chemicals used as host-finding stimuli by herbivores (Finch and Skinner, 1982). For example, aphids on squash plants were less abundant in maize-bean-squash polycultures than in squash monocultures. The plants in both systems had the same number of leaves, but the leaves were larger and older in squash monocultures because the shaded squash leaves in polycultures senesced more rapidly. Aphids were invariably found on the older leaves in both systems and reached very high densities on the oldest leaves in the monocultures. No very old leaves were in the polycultures, so aphids did not have the opportunity to reach the population densities that occurred in the monocultures (Andow and Risch, 1985). When a reduced attractiveness is due to the influence of the associate crop on the morphology of the host plants in intercrop system, it is likely to persist for some time after the removal of the associate by death or by harvest. Two whiteflies had lower egg densities on cassava mixed with cowpea than on cassava in monoculture with lower levels remaining in the intercrop for 6 months following cowpea harvest. Lower whitefly densities in multiple cropped systems during later stages of the cassava cycle resulted from effects of the intercrop on host-plant quality (Gold et al., 1990).

3.2.4 Resource concentration

To help explain the direct effects of vegetational diversity on specialist herbivores, Root (1973) proposed a resource concentration hypothesis which is adapted to consider the effects of intercrops on specialist herbivores. It states that herbivores will: (1) be less able to find their hosts because of visual and olfactory interference with their search pattern, (2) tend to stay for less time because of the disruptive effect of landing on non-host plants, and (3) have lowered survival and fecundity in diverse agricultural systems. The key idea was that the lower concentration of the host resource (and its dilution with non-host plants) will impose extra constraints on population growth. The resource concentration hypothesis predicts that specialist herbivorous insects should be more abundant in large patches of host plants, because they would find them more readily and stay there longer than in less concentrated host plant patches (Root, 1973). Some evidence supports this prediction (Kareiva, 1985, Bach, 1988, Sholes, 2008).

But, there is no agreement on the relative importance of immigration and emigration and determining the abundance of insects associated with patches of different sizes (Capman et al., 1990, Grez and González, 1995). The resource concentration hypothesis is organism-dependent, being a function of the adult and juvenile herbivore dispersal behavior in relation to the spatial scale of patchiness.

3.2.5 Natural enemies hypotheses

Maximizing survival and reproduction of beneficial arthropods requires provision of pollen and nectar resources that are often scarce in modern agricultural landscapes (Isaacs et al., 2008), and those resources could be provided by increasing biodiversity in agricultural system.

According to Root's natural enemies hypothesis, generalist and specialist natural enemies are expected to be more abundant in polycultures and therefore suppress herbivore population densities more in polycultures than in monocultures (Root, 1973). Identifying the key elements of diversity may be a difficult process, but the process can be guided by an understanding of the resources needed by natural enemies.

Generalist predators and parasitoids should be more abundant in polycultures than monocultures, and several possible reasons may contribute to this phenomenon: (1) they switch and feed on the greater variety of herbivores that become available in polycultures at different times during the growing season. (2) they maintain reproducing populations in polycultures while in monocultures only males of some parasitoids are produced. (3) they can exploit the greater variety of herbivores available in different microhabitats in the polyculture. (4) Finally, both generalist and specialist natural enemies should be more abundant in polycultures than monocultures because more pollen and nectar resources are available (Colley and Luna, 2000) at more times during the season in polycultures than monocultures. The amount of time available for predaceous carabid beetles to forage for prey was greater in polycultures than monocultures probably because polycultures had a moister, shadier soil surface microclimate, which enabled some of the beetles to forage during the day as well as at night (Brust et al., 1986).

The low incidence of pests in diverse agroecosystems has often been attributed to the higher abundance of their predators and parasites, because a greater range of available microhabitats, of alternative prey for unspecialised predators and parasites, and of nectar sources as supplements to the diet of parasites could be more available. The longevity of *Copidosoma koehleri* Blanchard, an important parasite of potato moth, *Phthorimaea operculella* (Zeller), was significantly increased when adults were caged on flowering plants of dill, borage, or coriander (Baggen and Gurr, 1998). Biodiversity could provide more shade, protection from desiccation by wind, lower mid-day temperatures, and other modifications of microhabitat (Altieri, 1999, Gurr et al., 2003). These modifications can affect herbivore movement and the activity of natural enemies (Andow, 1991).

The inconsistent opinions on the effects of biodiversity on specialist parasitoids were also proposed. Sheehan (1986) suggested that specialist parasitoids might be less abundant in polycultures than monocultures because chemical cues used in host finding will be disrupted and the parasitoids will be less able to find hosts to parasitize and feed upon in polycultures and the indistinct boundary at the edges of polycultures will be hard to recognize and they will be more likely to leave polycultural habitats than monocultures. In addition, Andow & Prokrym (1990) showed that structural complexity, or the connectedness of the surface on which a parasitoid searches, can strongly influence parasitoid host-finding rates; an implication is that structurally complex polycultures would have less parasitism than structurally simple monocultures.

The type of intercrop is likely to affect the relative importance of the resource concentration and natural enemies hypotheses. Where the intercrop provides a permanent vegetational cover, the interaction between pest and its enemies can more easily come into equilibrium, with outbreaks prevented. For this reason, biological control efforts are more successful in perennial crops than in annual crops (Trenbath, 1993). Where the associate species is an "insectary" plant, which by plentiful nectar production attracts herbivore predators and parasitoids, again the natural enemies hypothesis is more likely to be true (Atsatt and O'Dowd, 1976).

3.2.6 Diversity-stability hypothesis

The diversity-stability hypothesis states that the greater is the biological diversity of a community of organisms, the greater is the stability of that community (MacArthur, 1955, Elton, 1958, Pimentel, 1961). The diversity-stability hypothesis gained early acceptance based on its relevance to conservation and agriculture, including observations that monocultures in agricultural systems are prone to pest outbreaks and simpler island systems are more susceptible to species invasions (Andow, 1991). This hypothesis was tested by studying arthropod community dynamics in a long-term experimental manipulation of grassland plant species diversity. Over the course of a decade, higher plant diversity increased the stability of a diverse arthropod community across trophic levels. As the number of plant species increased, the stability of both herbivore and predator species richness and of total herbivore abundance increased (Haddad et al., 2011). The results show that higher plant diversity provides more temporally consistent food and habitat resources to arthropod foodwebs. Consequently, actively managing for high plant diversity may have stronger than expected benefits for increasing animal diversity and controlling pest outbreaks.

Tilman et al. (2006) presented the dependence of the temporal stability of ecosystems and species on plant diversity in a long-term grassland biodiversity experiment that established 168 plots containing 1-16 species. The results indicate that the reliable, efficient and sustainable supply of some foods, biofuels and ecosystem services can be enhanced by the use of biodiversity. As reviewed by Pimentel (1961), arthropod pest outbreaks could be decreased in diversity ecosystems due to the stability of community enhancing by higher plant diversity. Yet, the hypothesis has been a point of interest and debate for a half century (McNaughton, 1978, Tilman, 1996, Pfisterer and Schmid, 2002, Gross et al., 2009).

3.2.7 Associational resistance

Plants associated with taxonomically diverse plant species would suffer less herbivore attack than plants not so associated, Tahvanainen and Root (1972) called this phenomenon “associational resistance”. The associational resistance resulting from the higher taxonomic and microclimatic complexity of natural vegetation tends to reduce outbreaks of herbivores in diverse communities. Associational resistance has been well documented, and its mechanisms have been explored in tests of the resource concentration hypothesis (Connor et al., 2000). According to experimental data, Sholes (2008) pointed that specialist herbivores become less abundant when non-host species are mixed with their host plants and provided the evidence of associational resistance theory.

Neighboring plants could reduce herbivore damage (1) by their effects on the predator community, (2) by reducing the ability of herbivores to find their host plants, and (3) by reducing the time herbivores remain on their host plants. The abundance of the specialist herbivore *Galerucella calmariensis*, were affected by the presence of the nonhost *Myrica gale* (Hambäck et al., 2000). Hambäck et al suggested that the most likely mechanism causing decreased feeding on host plant was that *M. gale* affected the ability of *G. calmariensis* to find its host, either through visual or olfactory interference.

Associational resistance is also mediated by natural enemies. For instance, parasitism rates on *Iva frutescens* were higher on islands where

Borrichia frutescens co-occurred than on islands where *B. frutescens* were absent. Using both observations of natural communities and experimental manipulations, strong evidence was documented of an associational resistance mediated by natural enemies between *B. frutescens* and *I. frutescens* (Stiling et al., 2003). The associational resistance hypothesis has also many exceptions, and these cannot yet be accounted for (Andow, 1991).

3.2.8 Bottom-up and top-down forces

Host plants can impact herbivores directly by influencing their performance and survival, and indirectly by mediating the effects of natural enemies. Plant diversification can be beneficial to control pests via ‘top-down’ enhancement of natural enemy populations and by resource concentration and other ‘bottom-up’ effects acting directly on pests (Gurr et al., 2003). It is now generally accepted that bottom-up and top-down forces act in concert to influence populations of most phytophagous insects (Hunter et al., 1997, Gratton and Denno, 2003). Using a combination of time-series analysis of population counts recorded over 16 years and experimental data, Hunter et al. (1997) presented the first estimates of the relative roles of top-down and bottom-up forces on the population dynamics of two terrestrial insect herbivores on the English oak *Quercus robur*. Data suggested that spatial variation in *Operophtera brumata* density is dominated by host–plant quality. Just as habitat management can reduce pest attack by top-down effects operating via an enhancement of the third trophic level, pests may also be suppressed by bottom-up effects operating via the first trophic level of diverse habitats (Landis et al., 2000).

3.2.9 'Appropriate/inappropriate landings' theory

To explain why fewer specialist insects are found on host plants growing in diverse backgrounds than on similar plants growing in bare soil and why pest insects do not decimate wild host plants growing in 'natural' situations, the theory is based on the fact that during host plant finding the searching insects land indiscriminately on green objects such as the leaves of host plants (appropriate landings) and non-host plants (inappropriate landings), but avoid landing on brown surfaces, such as soil (Finch and Collier, 2000). In 2003, Field-cage experiments was carried out by Finch et al. showing that *Brassica* and *Allium* host-plants were each surrounded by four non-host plants to determine how background plants affected host-plant finding by the cabbage root fly *Delia radicum* L. and the onion fly *Delia antiqua* (Meig.) respectively (Finch et al., 2003).

The appropriate/inappropriate landing theory can be used to (1) explain why certain aspects of host plant finding by phytophagous insects, supposedly regulated by volatile plant chemicals, proved intractable in the past and (2)work equally well for generalist feeders, where the decision of whether to stay is determined primarily by the chemicals the insect detects via its contact chemoreceptors once it has landed on a leaf (Finch and Collier, 2000). Surely, the theory also needs more field and laboratorial evidences to confirm its effectiveness in future.

3.2.10 Push-pull or stimulo-deterrent diversion (SDD) strategy

Recently it has been observed that use of vegetative diversification, including intercropping and trap cropping, may hold potential to manipulate an agroecosystem in a push-pull or stimulodeterrent diversionary strategy. The term push-pull was first conceived as a strategy for insect pest management by Pyke et al. in Australia in 1987 (Pyke et al., 1987) in cotton system, thereby reducing reliance on insecticides. The concept was later formalized and refined by Miller & Cowles (1990), who termed the strategy stimulo-deterrent diversion (SDD) while developing alternatives to insecticides for control of the onion fly *D. antiqua*. In 2007, Cook et al. described the principles and components of the push-pull strategy, summarized developments over the past 20 years since the term was coined, and discussed how the strategy may contribute to addressing the global demand for the reduction of toxic materials in the environment as part of IPM strategies in the future(Cook et al., 2007). Push-pull strategy involves 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 (Cook et al., 2007). The strategy maximize efficacy of behavior manipulating stimuli through the additive and synergistic effects of integrating their use.

In biodiversity systems, push stimuli can be delivered by intercropping with nonhost plants that have repellent or deterrent attributes appropriate to the target pest. Kahn et al studied lepidopteran stem borers and the parasitoid *Cotesia sesamiae* (Cameron) in Africa. In this study, the grass *Melinis minutiflora* Beauv. produced volatiles that repel female stem borers and attract the foraging female parasitoids. Intercropping maize with this grass led to reduced infestation by the stem borer and increased rates of parasitism compared with a maize monoculture (Khan et al., 1997). Similar investigations were conducted for silverleaf desmodium *Desmodium uncinatum*, which released repellent HIPVs, were used as intercrops in a push-pull strategy for maize in Kenya (Khan and Pickett, 2004, Hassanali et al., 2008). This approach has recently been termed semiochemically assisted trap cropping (Shelton and Badenes-Perez, 2006) and also has been used in other plant-based push-pull strategy (Martel et al., 2005).

4. Habitat Management and Biological Control

The available literature suggests that the design of Habitat

Management strategies which improved biodiversity in agricultural system must include knowledge and consideration of (1) crop arrangement in time and space, (2) the composition and abundance of non crop vegetation within and around fields, (3) the soil type, (4) the surrounding environment, and (5) the type and intensity of management (Altieri, 1999). Thereby based on current ecological and agronomic theory, low pest potentials may be expected in agroecosystems that exhibit the following ways.

4.1 Diversification within a monoculture

Farmers tend to be risk-averse (Norton, 1976). This has led to some attempts to enhance pest management by making only subtle changes to normal management (Gurr et al., 2003). Strip-cutting of Lucerne *Medicago sativa* L. was tested as an alternative to the conventional practice of harvesting entire fields at a time (Hossain et al., 2001). In this system, natural enemies migrated from harvested strips into adjacent, un-harvested ones. When these refuges were cut some weeks later, natural enemies moved into the regrowing strips. Natural enemies exploit unharvested strips as refuges, and that enhancing the within-field community of natural enemies by strip harvesting contributes towards pest management.

4.2 Crop vegetation within-field

Greater levels of complexity in diversification may be adopted in crop vegetation within-field, in which one or more additional crop species are grown within the field, are used. This may take a variety of forms ranging in complexity from the simple inclusion of a discrete area of a secondary crop to complex spatial or temporal patterns of polycultures (Gurr et al., 2003).

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 (Trenbath, 1993, Ma et al., 2007), can also increase biodiversity in fields to encourage environmentally sustainable agricultural production with low inputs of pesticides (Ghaley et al., 2005). Cabbage was grown interplanted with several living mulches and in bare-ground monocultures in 1982 and 1983 at Freeville, N.Y.. Populations of *P. cruciferae* Goeze and *B. brassicae* (L.) were lower on cabbage grown with any living mulch than on cabbage in bare-ground monocultures (Andow et al., 1986). Hooks & Johnson (2001) interplanted broccoli, *Brassica oleracea* L. with chilli pepper *Capsicum annuum* L. or yellow sweetclover *Melilotus officinalis* L. suggesting that the latter treatment led to fewer Lepidoptera larvae in the broccoli heads compared with the chilli pepper or control treatments and the broccoli heads did not differ in size in Hawaii, USA. Cotton-wheat relay intercropping is practiced in northern China. The primary benefits are reduced damage by cotton aphid *Aphis gossypii* Glover on seedling cotton and increased productivity. Natural enemies are maintained in the field because they feed on prey in wheat and then easily disperse to emerging cotton seedlings where they can prevent population increase by *A. gossyppi* (Ma et al., 2006).

Another solution could be to diversify agroecosystems by increasing the number of species grown and using more leguminous crops (Altieri, 1999, Malézieux et al., 2009). In China，the maintenance of pea cover between rows of wheat crop reduced populations of insect pests *Sitobion avenae* (Fabricius) and enhanced the population and richness of natural enemies (Zhou et al., 2009a, Zhou et al., 2009b). Also, legume intercrops are also potential sources of plant nutrients that complement/supplement inorganic fertilizers by direct nitrogen transfer from the legume to cereal (Giller and Wilson, 1991). Additionally, the advantage of intercrops is that the two intercropped species do not compete for exactly the same resource niche and thereby tend to use resources in a complementary way (Hauggaard-Nielsen et al., 2001). Also crop mixing can contribute to enhance biodiversity as a similar approach to benefit the pest control in agricultural system. Weerapat et al (1977) found a reduction in the damage caused by brown plant hopper in mixtures of susceptible and resistant varieties of rice. At a site where the leafhopper was abundant, its population in the mixture was significantly smaller than the mean of observations in the sole crops at 5 out of 6 dates (Power, 1988).

The planting of attractive non-host "trap" crops as associates or as barriers around sole crops can reduce infestations of a susceptible crop. The use of such decoy plants has been carried further in the idea of a "protection" crop (Toba et al., 1977). This can be defined as an associate species that provides attractive feeding sites to the effective protection of the target crop. Another example of trap crop is the use of a lucerne strip within Australian cotton crops. The lucerne is ‘preferred’ over cotton by the green crop mired *Creontiades dilutus* (Stal), thus it also acts as a decoy or trap crop (Mensah and Khan, 1997). A dramatic field scale experiment demonstrated the efficacy of the trap cropping technique to protect larger areas of crop from pest by drilling white or black mustard *Sinapis alba* (L.) and pea *Pisum sativum* (L.) in the outer few meters of sweet corn *Zea mays* L. fields in New Zealand (Rea et al., 2002). The green vegetable bug *Nezara viridula* L. normally invades the crop from surrounding vegetation. In this study, the bugs remained in the mustard or pea, feeding on its developing pods, and this allowed the sweet corn to reach harvest stage with virtually no damage.

4.3 Biological corridor

The third way to reintroduce biodiversity into large-scale monocultures is by establishing vegetationally diverse field margins and/or hedgerows which may serve as biological corridors allowing the movement and distribution of useful arthropod biodiversity. A system of corridors can also have positive effects on the overall system by interrupting disease inoculum dispersion, by serving as barriers to insect pest movement, by modifying microclimate through interception of air currents, by influencing the flow of nutrients, materials and water and by providing habitat for wildlife. The most important function of corridors, however, arises through their manipulation, which can be an important first step in reintroducing biodiversity into large scale agroecosystems where natural vegetation patches have been virtually eliminated. In Europe, a variety of methods to enhance diversity at field edges have been introduced, including sown grass and flower strips (Marshall and Moonen, 2002), set-aside strips, borders of sown perennial vegetation (Marshall and Nowakowski, 1991) and conservation headlands, where the cereal crop edge receives reduced pesticide and herbicide inputs (Rands, 1985), and the impact of these on weed flora and arthropods indicate mostly beneficial effects though conflicts exist, notably for the conservation of rare arable weed species (Marshall and Moonen, 2002). Grass-sown in the centers of two cereal fields raised beetle banks have been used in British and mainland European arable crops for over a decade to provide overwintering habitat for natural enemies of aphid pests (Thomas et al., 1991, Wratten, 1992, Thomas et al., 2000, MacLeod et al., 2004). Comparisons of several grass species led to a recommendation to use cocksfoot *Dactylis glomerata* L. and Yorkshire fog *Holcus lanatus* L., perennials that have a dense tussock-forming growth habit and harbor the greatest numbers of predators (Thomas et al., 1992).

Removal of weeds generally through use of herbicides can be antagonistic to arthropod pest management. An alternative approach is to withhold all or some herbicides application in part of the crop and allow growth of the existing weed community. Those weeds may also favour natural enemies by providing non-host foods such as pollen and nectar, support non-pest alternative hosts or prey, and provide shelter or a moderated microclimate. This approach can apply also to perennial crop systems such as orchards and vineyards, where vegetational structure can include a distinct understorey. A considerable amount of work has taken place examining the effects of this relatively common form of diversification and it is particularly widely practised in China. The ground cover plant *Ageratum conyzoides* L. (Asteraceae) has been planted or conserved in 135000 ha of citrus where it is claimed to stabilise populations of *Amblyseius* spp., predators of the citrus red mite *Panonychus citri* McGregor (Liang and Huang, 1994). Weed strip management has been researched in Europe for several years (Landis et al., 2000). The practice involves establishing diverse mixtures of native plants in strips in and around fields. These strips have achieved a degree of acceptance in Swiss agriculture where they contribute to increased activity density of Carabidae (Coleoptera) (Lys et al., 1994), spiders (Araneida), Nabidae (Hemiptera), Dolichopodidae (Diptera) and Syrphidae (Diptera) (Hausmmann, 1996). Weed strip management appears to increase the availability of food for carabids and result in enhanced reproduction. Non-crop vegetation may be favored by natural enemies as oviposition sites. It has been observed that *Coleomegilla maculata* (Coleoptera: Coccinellidae) lays more eggs on a native weed *Acalypha ostryaefolia* Ridell than the sweet corn *Z. mays* L. crop, even though the plant supported few prey. Larvae then disperse from the weed and climb maize plants. Maize plots bordered by *A. ostryaefolia* contained significantly more *C. maculata* than did plots without a border (Cottrell and Yeargan, 1999). Borders of the flowering plant *Phacelia tanacetifolia* Bentham have been explored in cabbage *B. oleracea* L., where syrphid numbers increased, and aphid populations declined in New Zealand (White et al., 1995). Obviously, not all biological corridor in field can favour the natural enemy hypothesis to enhance the species richness, species abundance, or absolute abundance of spiders that reported by Chen et al. (2011b) through three years observations in tea plantations. The similar result was obtained for lady beetle in sorghum -wheat, alfalfa, and cotton relay-intercropping system (Phoofolo et al., 2010).

Field margins are a key feature of agricultural landscapes, present in some form at the edges of all agricultural fields (Marshall, 1988), which contribute to the sustainability of production, by enhancing beneficial species within crops and reducing pesticide use. The biodiversity of the margin may be of particular importance for the maintenance of species at higher trophic levels, notably farmland birds, at the landscape scale (Marshall and Moonen, 2002). There is wide acceptance of the importance of field margins as reservoirs of the natural enemies of crop pests. Many studies have demonstrated increased abundance of natural enemies and more effective biological control where crops are bordered by wild vegetation. These habitats may be important as overwintering sites for natural enemies and may provide increased resources such as alternative prey/hosts, pollen and nectar for parasitoids and predators from flowering plants (Landis, 1994). A field trial found that rates of parasitism were greater among *P. operculella* larvae recovered from potato plants growing close to a strip of flowers than in larvae 20m distant, suggesting that there may be value in providing nonhost foods to *C. koehleri* by deploying flowering plants (Baggen and Gurr, 1998). The similar effect was also observed for hoverflies using *Phelia tanacetifolia* strips to enhance biological control of aphids in wheat fields (Hickman and Written, 1996).

4.4 Adjacent plants

Given the high edge-to-area ratio in the margins, these features are expected to have a high degree of interaction with adjacent plants, thereby providing protection against insect pests within the area of influence of the corridors. At a greater level of complexity, changes may be made that apply beyond the field boundary at a larger spatial scale(Gurr et al., 2003).

A mix of perennial flowering plants was grown adjacent to corn *Z. mays* L. to provide supplementary food for predators and parasitoids, two-year data showed that the flowering plants buffer the negative consequences of insecticide application on carabids in adjacent fields (Lee et al., 2001). The effectiveness of adjacent crop rape was also observed for conserving natural enemies of aphids in wheat field in China (Fei et al., 2011). The presence of old field adjacent strips along rape fields was associated with increased mortality of pollen beetles resulting from parasitism and adjacent, large, old fallow habitats had an even greater effect, providing evidence that complex landscapes with a high density and connectivity of uncultivated, perennial habitats may enhance populations of natural enemies, which immigrate into neighboring annual crop fields, attack pest insects, and contribute significantly to the reduction of pest populations below an economic threshold (Thies and Tscharntke, 1999). Additionally, however, tall boundary vegetation, such as trees, may impede hoverfly dispersal into nearby areas of crop (Wratten et al., 2003). Thus, habitat structure may constrain the spatial extent of the benefits from adding floral resources to existing boundaries. Potentially extending beyond the farm boundary, features such as areas of woodland and hedgerow, can have a long-range effect on pest management.

Different options to diversify cropping systems are available depending on whether the current monoculture systems to be modified are based on annual or perennial crops. Rotation, interplant and multiple cropping systems are effective management strategies for annual monocultures. In the case of perennial crops, research suggests that cover cropping transforms orchards and vineyards into agroecosystems of increasing ecological diversity and stability. Systematic studies on the appropriate combination of plant diversification with respect to the abundance and efficiency of natural enemies are needed. The above generalizations can serve in the planning of a vegetation management strategy in agroecosystems. However, they must take into account local variations in climate, geography, crops, local vegetation, inputs, pest complexes, and so on, which might increase or decrease the potential for pest development under certain vegetation management conditions. The selection of component plant species can also be critical.

5. Conclusion

Diversity in agroecosystems may favor reduced pest pressure and enhanced activity of natural enemies. However, several authors have noted that to selectively enhance natural enemies, the important elements of diversity should be identified and provided rather than encouraging diversity per se (Southwood and Way, 1970, van Emden and Williams, 1974, Speight, 1983). Indeed, it has been shown that simply increasing diversity can exacerbate certain pest problems. During the winter season, the average number of lepidopteran contaminants per broccoli head was more than twice that in monoculture and pepper intercropped broccoli than in broccoli-YSC habitats (Hooks and Johnson, 2001). These effects of diversification can only be determined experimentally across a whole range of agroecosystems. The task is indeed overwhelming since enhancement techniques must necessarily be site specific.

In spite of the some contradictions encountered, this review has summarized some systems in which insect pest impact has been regularly reduced through diversification of agricultural systems. It is concluded that the pest management potential of biodiversity is variable and dependent on environmental factors, but it is recommended that biodiversity be used in integrated pest management systems with the progressive decrease in insecticide use. The response of insect populations to environmental manipulations depends upon their degree of association with one or more of the vegetational components of the system (Altieri, 1999). Biodiversity performs key ecological services and if correctly assembled in time and space can lead to agroecosystems capable of sponsoring their own soil fertility, crop protection and productivity (Altieri, 1999). Correct biodiversification results in pest regulation through restoration of natural control of insect pests, diseases and nematodes and also produces optimal nutrient recycling and soil conservation by activating soil biota, all factors leading to sustainable yields, energy conservation, and less dependence on external inputs (Altieri, 1999).

Finally, increasing biodiversity will normally be complemented by other methods and should not be promoted as a standalone method. Commonly these will employ biological control agents that have been released in classical or augmentative manners. In such instances habitat management holds considerable potential for enhancing the success rates of classical agents, and to maximize the persistence and impact on pest population of augmentative agents. In the future, these formerly separate branches of biological control will be merged to synergistic effect in “integrated biological control”.

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Table1. Biological parameters of herbivores influenced by agricultural diversification of crops

crop	**Companion plant**	**Insect species**	**Country**	**Reference cited**
Wheat	Pea	*Sitobion avenae*	China	(Zhou et al., 2009a, Zhou et al., 2009b)
Wheat	Pea	*Metopolophium dirhodum*	Pakistan	(Ehsan and van Emden, 2003)
	Rape			
Wheat	Garlic	*Sitobion avenae*	China	(Wang et al., 2009, Wang et al., 2011)
Wheat	Alfalfa	*Sitobion avenae*	China	(Ma et al., 2007)
		Rhopalosiphum padi		
		Sitobion avenae		
Wheat	Alfalfa	*Schizaphis graminum*	USA	(Hesler et al., 2000)
Wheat	Alfalfa	*Meormyza americana*	USA	(Hesler and Kieckhefer, 2000)
Maize	Sorghum	*Busseola fusca*	Kenya	(Khan et al., 2000)
Maize	Sudan grass	*Chilo partellus*	Kenya	(Khan et al., 2001)
		Chilo partellus		
		Chilo orichalcociliellus		
Maize	Cowpea	*Sesamia calamistis*	Denmark	(Skovgard and Pats, 1996)
		Busseola fusca		
Maize	Sorghum	*Chilo partellus*	South Africa	(Van den Berg et al., 2001)
	Millet			
	Bean			
Maize	Sorghum	*Chilo partellus*	Kenya	(Songa et al., 2007)
Maize	Cassava	*Sesamia calamistis*	Republic of Benin:	(Schulthess et al., 2004)
	Hedgerow			
Maize	Woodlot	*Pseudaletia unipuncta*	USA	(Marino and Landis, 1996)
		Aphis gossypii		
Cotton	Wheat	*Sitobion avenae*	China	(Xia, 1997, Ma et al., 2006)
Cotton	Basil	*Pectinophora gossypiella*	Egypt	(Schader et al., 2005)
Cotton	Alfalfa	*Aphis gossypii*	China	(Chen et al., 2011c)
	Canola			
	Wheat			
Cotton	Sorghum	*Aphis gossypii*	USA	(Parajulee et al., 1997)
		Aphis craccivora		
		Megalarothrips sjostedi		
Cowpea	Sorghum	*Maruca vitreta*	Nigeria	(Hassan, 2009)
Broad bean	Basil	*Aphis fabae*	Germany	(Basedow et al., 2006)
Snap bean	Maize	*Epilachna varivestis*	USA	(Coll and Bottrell, 1994)
	Alyssum			
	Phacelia			
Apple	Buckwheat	*Epiphyas postvittana*	New Zealand	(Irvin et al., 2006)
		Brevicoryne brassicae		
Brussels sprout	French beans	*Delia radicum*	Uganda	(Tukahirwa and Coaker, 1982)
Broccoli	Chili pepper	*Artogeia rapae*	USA	(Hooks and Johnson, 2006)

	Yellow sweet clover	*Trichoplusia ni*		
	Tomato			
	Pepper			
Cabbage	Onion	*Plutella xylostella*	Ghana	(Mohammed et al., 2010)
		Brevicoryne brassicae		
		Myzus persicae		
Cabbage	Lacy phacelia	*Plutella xylostella*	New Zealand	(White et al., 1995)
Canola	Wheat	*Phyllotreta* spp	Canada	(Hummel et al., 2009)
Canola	Wheat	*Aleochara bilineata*	Canada	(Hummel et al., 2010)
		Psila rosae		
Carrot	Onion	*Thrips tabaci*	UK	(Uvah and Coaker, 1984)
Collard	Potato	*Phyllotreta cruciferae*	USA	(Bergelson and Kareiva, 1987)
		Psylla chinensis		
		Aphis citricola		
Pear	Aromatic plants	*Pseudococcus comstocki*	China	(Song et al., 2011)
		Liriomyza huidobrensis		
Pepper	Sugarcane	Becker	China	(Chen et al., 2011a)
	Wheat	*Clostera fulgurita*		
Poplar	Barseem	*Clostera restitura*	India	(Sangha, 2011)
Strawberry	Wheat	*Agriotes obscurus*	Canada	(Vernon et al., 2000)
		Mamestra brassicae		
		Brevicoryne brassicae		
white cabbage	Clover	*Delia brassicae*	Netherlands	(Theunissen et al., 1995)
	Buckwheat			
	White clover			
	Sunn hemp			
Zucchini	Okra	*Bemisia argentifolli*	USA	(Manandhar et al., 2009)

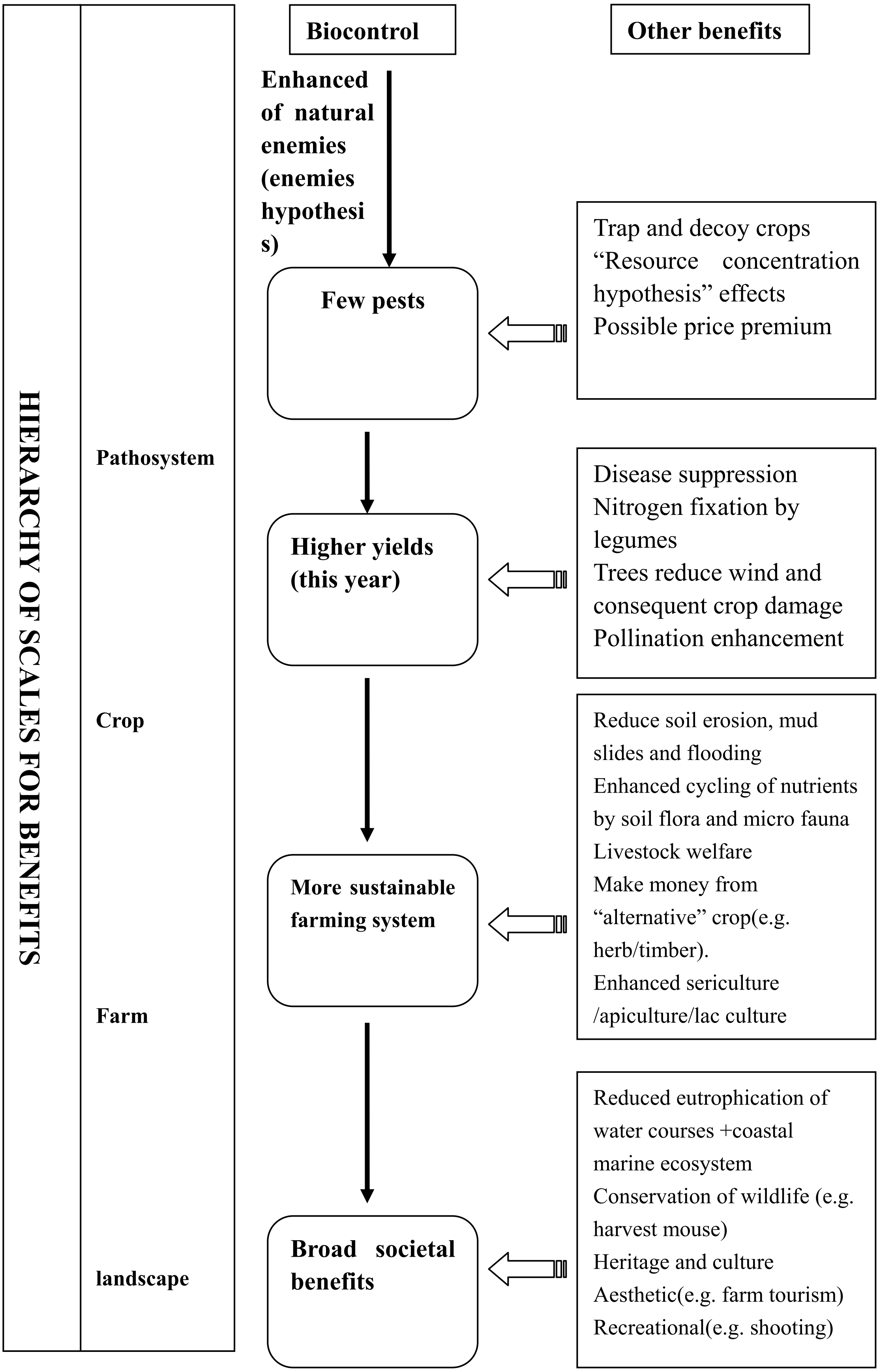

Fig.1 The hierarchy of scale for potential benefits of multi-function agricultural biodiversity (Gurr, et. al. 2003)

Chapter III: *OBJECTIVES*

The aim of this thesis was to promote the use of the intercropping and infochemical releasers as Integrated Pest Management (IPM) agents by developing alternative strategies for aphid control. The first objective was to evaluate the resistance to aphids of wheat germplasm lines and develop the approaches and strategies for structuring fuzzy recognition technique. The second objective was to promote the use of infochemicals, emanating from plants or aphids, as the alternative strategy that benefit natural enemies conservation and aphids decline. The last objective was to expanded the adaptation of wheat-pea intercropping pattern in China and Belgium to reduce cereal aphid occurrence by promoting natural enemies by increasing biodiversity in wheat farming system.

In the fourth chapter of this thesis, we were beginning to screen and evaluate the resistance to aphids of wheat germplasm lines in three wheat-producing areas of China, the approaches and strategies for structuring fuzzy recognition technique in evaluation on aphid-resistant wheat germplasm lines was also discussed.

In the fifth chapter of this thesis, Extensive evidences imply that nearly all herbivorous insects and their natural enemies can perceive and positively respond to plant volatiles. In the present investigation, (E)-β-farnesene, garlic extraction and (Z)-3-hexenol were released in wheat crop. The objective was to assess the potential of those volatiles on aphid management strategy by reducing the preference of aphids and preserving their natural enemies.

Also, we compared the effects of wheat monoculture, wheat-garlic intercropping (wheat cultivars with different resistant levels to wheat aphids), treatment with a garlic oil blend, and diallyl disulfide release in wheat fields on *S. avenae*, their natural enemies, and overall crop yield.

Finally, in the last chapter of this thesis, to understand the mechanisms by which diversification of habitat may favor pest management, we tested the impact of associating pea to wheat in several combinations (1) on behavioural preference of one aphid pest, namely *S. avenae* and (2) on aphidopagous beneficials *H. axyridis* and *E. balteatus.*

Base on the conclusion of beneficial effect of associating pea to wheat in laboratory, the field experiments of wheat-pea intercropping or mixing were performed in China and Belgium. We assessed the effect of flowering plant as buffer strips in wheat fields on the populations of aphids and their natural enemies, to determine whether this form of habitat management would provide a flowering plant as an alternative strategy for enhancing abundance of natural enemies to benefit the conservation biological control.

Chapter IV: *EVALUATION ON THE RESISTANCE TO APHIDS OF WHEAT GERMPLASM RESOURCES IN CHINA*

General Introduction to Chapter IV

Host plant resistance plays important roles in controlling pests and protecting of natural enemies in an agroecosystem. The attributes that often enhance aphid's predator effectiveness and directly stress aphid population development may be genetically varied among plants. Plant resistance to insects often affects individual development, fecundity and population growth of insects by secondary plant substances. Athough the analysis of why plants are resistant indicates that three basic components are nonpreferred, antibiosis and tolerant, the large number of aphids supported by resistant seedlings in greenhouse screening tests indicates that a major component of resistance in these germplasm lines is tolerance. The application of resistant varieties could be regard as one of the most effective approach in aphid biological control in agricultural systems.

Biotypes, the presence of biological strains of insects, constitute an important feature of the environment that may modify the expression of resistance, and such biotypes may occupy definite geographic areas. Seedlings in greenhouse flats have ample moisture and nutrients as well as favorable temperatures and are not exposed to natural stresses that may occur in the field during any growing season and which could impact the expression of resistance, and aphids are protected from exposure to parasites and predators compare in the field, as well as wind and rain. As a result, aphids build up to great numbers even on flat leaves of resistant seedlings. So the evaluation based on seedlings in greenhouse could be susceptibility. For those reasons that we were beginning to screen and evaluate the resistance to aphids of wheat germplasm lines in three wheat-producing areas of China.

Evaluation on the resistance to aphids of wheat germplasm resources in China

Haibo Zhou[a,b], Julian Chen[a], Dengfa Cheng[a], Frédéric Francis[b], Yong Liu[c], Jingrui Sun[a], Ying Huang[d], Xiaosong Wang[d], Xingwang Liu[d], Liu Xiaoming[d] and Jiandong Zeng[e]

[a] State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China.

[b] Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux, 5030, Belgium.

[c] College of Plant Protection, Shandong Agricultural University, Taian, 271018, PR China.

[d] Mianyang Agricultural Bureau, Mianyang 621000, PR China.

[e] Jiangyou Agricultural Bureau, Jiangyou 621700, PR China.

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Abstract: A collection of more than 200 wheat lines from main wheat-producing areas of China was evaluated for resistance to wheat aphids using fuzzy recognition technique in five field experiments over 2 years. The results showed that susceptible to wheat aphids was exhibited in most of the lines tested, and no immune and highly resistance lines to wheat aphids was observed. The average percentage of wheat germplasm lines with resistant, lowly susceptible, moderately susceptible and highly susceptible to aphid were 9.30%, 23.15%, 42.32%, and 25.23%, respectively. 5 moderately resistant wheat germplasm lines to wheat aphids (Lantian18, Lantian20, Lantian22, Lantian00-30 and Shanmai175) were found in Jiangyou experimental station in 2009. More importantly, 2 wheat germplasm lines (Lantian20, Lantian22) with the continuous resistance to wheat aphid in the five experimental stations over 2 years were discovered. Although resistance of wheat germplasm lines had a close relation to their genetics and inheritance, we also found that the resistance of the same wheat germplasm lines was varied in different experimental stations. It would be helpful to make wheat germplasm selections for breeding programs, especially if they have unique genes that may provide resistance to future biotypes of wheat aphids. A valuable method for evaluating the potential of aphid-resistance for wheat germplasm lines was also confirmed.

Key words: wheat germplasm lines; aphid; resistance identification

1.Introduction

Sitobion avenae (Fabricius), *Schizaphis graminum* (Rondani), and *Rhopalosiphum padi* (Linnaeus), the dominant and destructive pests in wheat production regions of China (Ma et al., 2006; Wang et al., 2009; Zhao et al., 2009), can cause heavy economic damage to wheat both as a phloem feeder and as a vector of plant viruses (Quillec et al., 1995; Van Emden and Harrington, 2007; Liu et al., 2009; Ma et al., 2010).

To avoid environmental pollution and health problems caused by the overuse of traditional synthetic pesticides, exploration of host plant resistance to pest management is a necessary research theme in sustainable agriculture system. Host plant resistance plays important roles in controlling pests and protecting of natural enemies in an agroecosystem (Francis et al., 2001; Messina and Sorenson, 2001), and the effect on application of insect-resistance plant varieties in reducing pest damage is considered to be conspicuous (Painter, 1958). A field study of Russian wheat aphid on yield and yield components of field grown susceptible and resistant spring barley in Laramie showed highly resistant lines maintained or increased yield components and grain yield (average grain yield increase 5%) under aphids feeding pressure, and susceptible cultivars had a large reduction in yield components and grain yield (average reduction 56%) (Mornhinweg et al., 2006). In assessing the effect of a resistant variety on an insect population, the literature suggests that the effect is likely to be cumulative. Three times as many pea aphids in the field on susceptible varieties as on resistant ones was found in each year during a nine-year study (Maltais, 1951). Brewer et al. also reported that *D. noxia* abundance on resistant barley lines was lower than that on more susceptible lines (Brewer et al., 1999). In a separate field study, the host plant resistance against aphids enhanced the parasitism of aphid species *Sitobion avenae* (F.) by its parasitoid *Aphidius* spp. in wheat field (Cai et al., 2009).

The evaluations on identification of resistance to cereal aphids in Wheat germplasm lines have also been studied. Smith et al, working with the Russian wheat aphid, identified five new sources of low levels of resistance (PI 47545, PI 94355, PI 94365, PI 94460, and PI 151918) from Iran and the Soviet Union in three breeding lines from Idaho, one breeding line from Texas (Smith et al., 1991). About 8 wheat varieties have been identified as Cereal aphids- resistant wheat germplasm lines over a five-year field study from 577 varieties in Henan province, China (Li et al., 1998).

Seedlings in greenhouse flats have ample moisture and nutrients as well as favorable temperatures and are not exposed to natural stresses that may occur in the field during any growing season and which could impact the expression of resistance (Mornhinweg et al., 2006), and aphids are protected from exposure to parasites and predators compare in the field, as well as wind and rain. As a result, aphids build up to great numbers even on flat leaves of resistant seedlings. So the evaluation based on seedlings in greenhouse could be susceptibility. Biotypes, the presence of biological strains of insects, constitute an important feature of the environment that may modify the expression of resistance, and such biotypes may occupy definite geographic areas. For those reasons that we were beginning to screen and evaluate the resistance to aphids of wheat germplasm lines in three wheat-producing areas of China, the approaches and strategies for structuring fuzzy recognition technique in evaluation on aphid-resistant wheat germplasm lines was also discussed.

2. Materials and methods

Experimental field and wheat varieties

The experiment was conducted during two seasons (2009 and 2010) at Langfang, Hebei province in Northern Wheat Region, Jiangyou, Sichuan province in Southwestern Wheat Region and Xinxiang, Henan province in Huan-Huai-Hai Wheat Region, sites representing diverse environments in China. Langfang, at 20 m above sea level (m a.s.l.), represents the warm temperate continental monsoon climate with 554.9 mm annual rainfall. Jiangyou at 510 m a.s.l. is in the humid subtropical monsoon climate and receives 859.9 mm of rainfall. Xinxiang, at 75 m a.s.l., also represents the warm temperate continental monsoon climate with 656.3 mm annual rainfall.

More than 200 wheat germplasm lines recommended from Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Northwest A&F University, and academy (institution) of agricultural sciences of some provinces and cities in China were evaluated in field. And susceptible to cereal aphids variety, c.v. Beijing 837 was planted as control variety (CV).

Methods

The experiment was conducted as described in the rules for resistance evaluation of wheat to diseases and insect pests, Part 7：Rule for resistance evaluation of wheat to aphids, Agriculture industry standard of the People's Republic of China (NY/T 1443.7-2007).

Nursery of resistance evaluation

The wheat was sown in drill in the nursery (250cm border width, 50cm border dike width) as sketch map in Fig. 1, and the length of the nursery depended on the terrain of cultivated area.

Fig1. The sketch map of nursery for evaluation in field

The evaluation for each variety was laid out in a randomized complete block design with three replications. Two rows, 1-m-long for every wheat line and 1 CV in every 9 varieties were planted at a spacing of 0.3 m between rows. In order to attract more aphids, the CV was also planted in line in and around the field. Wheat was sown at rates to provide 100 seeds per 1-m-long row in all varieties. No pesticides and herbicides were applied on the fields during the entire growing season.

Investigation method of wheat aphids

In gain-filling stage of most wheat lines, the high occurrence period of cereal aphids, the levels infested by cereal aphid metapopulation including *Sitobion avenae* (Fabricius), *Schizaphis graminum* (Rondani), *Rhopalosiphum padi* (Linnaeus), *Metopolophium dirhodum* (Walker) were recorded using fuzzy recognition technique by 6 regular investigators divided 3 groups. The wheat infested with most abundant aphids was selected to be as the criterion of the wheat variety, and the rating scale infested by wheat aphids is presented in table 1.

Table 1. Rating scale infested by wheat aphids

The evaluation index (R)

The R index, a parameter to evaluate the resistance to wheat aphid for wheat varieties was presented in Table 2.

$$\hat{\mathbf{I}} = \frac{\sum_{i=1}^{n}\left(M_{i1} + M_{i2} + M_{i3}\right)}{3n} \qquad R = \frac{I}{\hat{\mathrm{I}}}$$

Where M is mode of the level of rating scale infested by wheat aphid for each replication, n is the total of wheat varieties and I is the maximum value of mode for each wheat varieties in three replications.

Table.2 The evaluation index (R) of resistance to cereal aphids

3. Results

Table 3. The result for evaluation of resistance to aphids of wheat germplasm lines in two years

The results for evaluation of resistance to cereal aphids in two years are presented in Table 3 and Table 4. There were 29 and 24 wheat varieties with resistance to cereal aphids in Jiangyou and Langfang respectively in 2009; 24, 23 and 10 wheat varieties with resistance to cereal aphids were observed in Jiangyou, Langfang and Xinxiang respectively in 2010. Most of wheat varieties with resistance to aphid in this evaluation were lowly resistant except for 5 wheat varieties (Lantian18, Lantian20, Lantian22, Lantian00-30 and Shanmai175) with moderately resistant in Jiangyou in 2009. The average percentage of the wheat germplasm lines with resistant, lowly susceptible, moderately susceptible and highly susceptible to cereal aphids were 9.30%, 23.15%, 42.32% and 25.23% in entire wheat germplasm resources respectively.

Table 4. The varieties of resistance to cereal aphids in two years

Table 5. The consistent wheat varieties with resistance to cereal aphids in two years

The comparative study of wheat varieties with resistance in the one location showed several wheat varieties displayed consistent resistance to cereal aphids in 2 years (Table. 5). The consistent wheat varieties in Sichuan were Lantian-18, Lantian-20, Lantian-22, Mianmai-37, Mianmai-185, Hanmai-111, Linzao51329 and Changwu134; and in Hebei, 7 wheat varieties, including Mianmai37, Maimian39, Ningmai13, Lantian17, Lantian20, Lantian21 and Lantian22, also displayed consistent resistance to cereal aphids. We also found 2 wheat varieties, Lantian20 and Lantian22 possessed resistance to cereal aphids in the 5- evaluation test in the field.

4. Disscussion

The widespread development of resistance to many of these insecticides by pest species has caused thoughtful entomologists to realize that all possible means must be employed in insect control (Painter, 1958). The analysis of why plants are resistant indicates that three basic components are nonpreferred, antibiosis and tolerant, and two reasons could explain why resistant plant can reduce the damage by insect: (1) Plant resistance to insects often affects individual development, fecundity and population growth of insects by secondary plant substances, but can not result in insect mortality (Cai et al., 2009). (2) The attributes that often enhance aphid's predator effectiveness and directly stress aphid population development may be genetically varied among plants (Rutledge et al., 2003; Kagata et al., 2005). It could provide a more economical, timely and efficient strategy using plant resistance as a pest control method in agroecosystem. And host plant resistance offers the only cost effective means of cereal aphids control. In this study, we found that the majority of wheat germplasm resources were evaluated as susceptible to cereal aphids, and no immune and highly resistant variety was observed. 2 wheat varieties (Lantian20 and Lantian22) with the continuous resistance to cereal aphids in the five experimental fields over 2 years were found.

Germplasm must be evaluated for useful traits before it can be fully utilized (McCarty et al., 1998). Evaluations, such as the one reported here, aid plant breeders in making germplasm selections for breeding programs, especially if they have unique genes that may provide resistance to future biotypes of cereal aphids. These evaluations on reaction to aphid metapopulation that could be crucial when germplasm is used in improving production and qualities of wheat cultivars. This research is part of program to evaluate germplasm for useful traits and make this information available to the germplasm system. The resistance of these identified lines of wheat here awaits further confirmation of the expression. Future searches for aphid-resistant germplasm should concentrate to the genetics and inheritance of aphid resistance in these new sources.

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Figures and tables

Figure

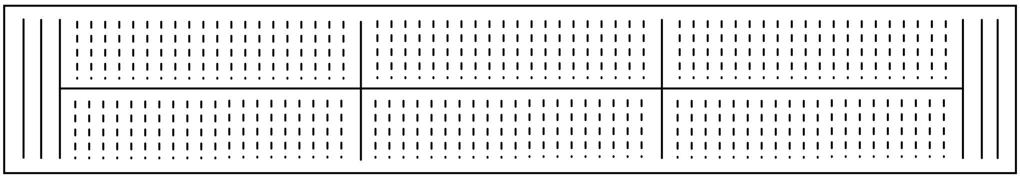

Fig1. The sketch map of nursery for evalution in field. □: Border dike; ——:Control wheat variety; ——: Evaluated wheat varieties.

Tables

Table 1. Rating scale infested by wheat aphids

Level	Rating scale of aphids in one plant
0	None
1	Less than 10 aphids
2	10-20 aphids，wheat ear infested with none or 1-5 aphids
3	21-50aphids, wheat ear infested with 6-10 aphids
4	More than 50 aphids, one-fourth of wheat ear infested with aphids
5	One-fourth to three fourth of wheat ear infested with aphids
6	The whole plant infested with aphids

Table.2 The evaluation index (R) of resistance to cereal aphids

Resistance level	R	Resistance to wheat aphid
0	0	Immune（I）
1	0.01～0.30	Highly resistant（HR）
2	0.31～0.60	Moderately resistant（MR）
3	0.61～0.90	Lowly resistant（LR）
4	0.91～1.20	Lowly susceptible（LS）
5	1.21～1.50	Moderately susceptible（MS）
6	>1.50	Highly susceptible（HS）

Table 3. The result for evaluation of resistance to aphids of wheat germplasm lines in two years

Year	Location	MR	P%*	LR	P%	LS	P%	MS	P%	HS	P%	T**
2009	Jiangyou.Sichuan	5	2.36	24	11.32	33	15.57	64	30.19	86	40.57	212
	Langfang.Hebei	0	0	24	11.32	53	25.00	135	63.68	0	0	212
2010	Jiangyou.Sichuan	0	0	24	9.16	86	32.82	73	27.86	79	30.15	262
	Langfang.Hebei	0	0	23	8.07	33	11.58	110	38.60	119	41.75	285
	Xinxiang.Henan	0	0	10	4.27	72	30.77	120	51.28	32	13.68	234
	Mean		2.36		8.83		23.15		42.32		25.23	

Note: *percentage of the total wheat varieties, ** The total wheat varieties

Table 4. The varieties of resistance to cereal aphids in two years

Jiangyou.Sichuan				Langfang.Hebei				Xinxiang.Henan	
2009		2010		2009		2010		2010	
Varieties	RCA*	Varieties	RCA	Varieties	RCA	Varieties	RCA	Varieties	RCA
Lantian18	MR	Lantian18	LR	Mianmai37	LR	Mianmai37	LR	Mianmai185	LR
Lantian20	MR	Lantian20	LR	Mianmai39	LR	Mianmai39	LR	Xikemai5	LR
Lantian22	MR	Lantian22	LR	Mianmai45	LR	Mianmai46	LR	Lantian20	LR
Lantian00-30	MR	Xikemai4	LR	Xikemai2	LR	Mianmai185	LR	Luohan8-1	LR
Shanmai175	MR	Yumai52	LR	Xikemai4	LR	Hanmai111	LR	Mianmai39	LR
Zhoumai17	LR	Yunong035	LR	XK0106-108D6	LR	Ningmai13	LR	Lantian17	LR
Aikang58	LR	Zhoumai16	LR	Beijing0045	LR	Zhoumai18	LR	Mianmai46	LR
Mianmai37	LR	Yan2415	LR	Een1	LR	Lantian17	LR	Lantian21	LR
Mianmai45	LR	Yan5158	LR	Emai12	LR	Lantian20	LR	Lantian22	LR
Mianmai185	LR	Lantian15	LR	Emai23	LR	Lantian21	LR	Lantian23	LR
Xikemai2	LR	Mianmai37	LR	Huamai8	LR	Lantian22	LR		
Xikemai5	LR	Youmai8004	LR	Ningmai13	LR	Youmai8004	LR		
Hanmai111	LR	Mianmai185	LR	Yannong19	LR	Linmai4	LR		
Emai16	LR	Chang6359	LR	Zhenmai5	LR	Wenqian(4)1	LR		
Huaimai17	LR	Lunong116	LR	Zhengmai004	LR	Xinong9871	LR		
Ningmai13	LR	Hanmai111	LR	Lantian15	LR	Yang06-144	LR		
Zhoumai22	LR	Hengguan111	LR	Lantian17	LR	Yunong202	LR		
Yannong24	LR	Linyou2618	LR	Lantian20	LR	Guan0014	LR		
Lantian99-316	LR	05-83	LR	Lantian21	LR	70222-24	LR		
Lantian21	LR	Lantian21	LR	Lantian22	LR	Neimai8	LR		
Lin867	LR	Linzao51329	LR	Zhongnong2	LR	Mian06-367	LR		
Changhan58	LR	Mianmai46	LR	Ningdong10	LR	Mian06-374	LR		
Linzao51329	LR	Mianmai39	LR	Shan715	LR	Mian1971-98	LR		
Luohan7	LR	Changwu134	LR	Luohan7	LR				
Luohan8-1	LR								
Xinong889	LR								
Xinong3517	LR								
Changwu134	LR								
Hengguan136	LR								

Note: * RCA=Resistance to cereal aphids

Table 5. The consistent wheat varieties with resistance to cereal aphids in two years

Jiangyou. Sichuan.				**Langfang. Hebei**			
2009		2010		2009		2010	
Varieties	RCA	Varieties	RCA	Varieties	RCA	Varieties	RCA
Lantian-18	MR	Lantian18	LR	Mianmai37	LR	Mianmai37	LR
Lantian-20	MR	Lantian20	LR	Maimian39	LR	Maimian39	LR
Lantian-22	MR	Lantian22	LR	Ningmai13	LR	Ningmai13	LR
Mianmai-37	LR	Maimai37	LR	Lantian17	LR	Lantian17	LR
Mianmai-185	LR	Mianmai185	LR	Lantian20	LR	Lantian20	LR
Hanmai-111	LR	Hanmai111	LR	Lantian21	LR	Lantian21	LR
Linzao51329	LR	Linzao51329	LR	Lantian22	LR	Lantian22	LR
Changwu134	LR	Changwu134	LR				

Chapter V: *THE POTENTIAL OF INFOCHEMICALS IN INTEGRATED PEST MANAGEMENT*

General Introduction to Chapter V

Host-plant resistance involves modifying some anatomical, morphological, physiological, or chemical attribute of the plant. There is therefore always the possibility that this will make the plant more susceptible to another damaging organism. Host-plant resistance has far greater potential for reducing populations of aphids than has as yet been exploited. This is partly because plant breeders have sought to use host-plant resistance as a single-component control measure.

Chemical pesticides have been a boon all over the world, especially in developing countries in their efforts to eradicate insect-borne, endemic diseases, to produce adequate food and to protect crops. Controversy exists over the global dependence on such agents, given their excessive use or misuse, their volatility, long-distance transport and eventual environmental contamination in colder climates. In the 1970's the World Health Organisation (WHO) estimated that there were globally 500,000-pesticide poisonings per year, resulting in 5,000 deaths. Therefore, alternative stratehies of pest contol are desired relevant to maintain or improve crop`s productivity and sustainability.

Semiochemicals from aphids, host and non-host plants convey information that is vital for selecting feeding, larviposition, attracting a mate, aggregating with conspecifics, avoiding competition and sensing or giving warning of threats. The volatile semiochemicals may be produced in defense against herbivores but may also serve a secondary function in attracting the natural enemies of these herbivores. Due to their potential alternatives as a biological control agent against wheat aphid, garlic intercropping and related emitted volatiles are expected to contribute to the further improvement of integrated pest management systems and to potentially reduce the amount of traditional synthetic pesticides applied in wheat fields.

V.1. Use of plant infochemical slow releasers to control aphids: a first investigation in Belgian wheat field

Haibo Zhou(1,2), Julian Chen(1), Yong Liu(3), Eric Haubruge(2), Dengfa Cheng(1), Frédéric Francis(2)

(1) State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China

(2) Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux, 5030, Belgium

(3) College of Plant Protection, Shandong Agricultural University, Taian, 271018, PR China

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Abstract

Use of infochemicals to develop push-pull strategy in pest control is a potential way to promote sustainable crop production. Field experiments were performed in wheat exploiting infochemicals from plant essential oils in slow releasers to control population density of cereal aphids and to promote their natural enemies. *Metopolophum dirhodum* and *Sitobion avenae* were the predominant species on wheat. (Z)-3-hexenol (Z3H) attracted aphids and should be considered as useful infochemical in aphid control by promoting attraction of aphids outside field plot. Releases of (E)-β-farnesene (EBF) and garlic extraction (GE) allowed to significantly decrease the abundance of wheat aphids. The main natural enemies of cereal aphids were the lacewings (47.8%), the hoverflies (39.4%), and ladybirds (12.8%). Significant higher abundances of hoverflies and lacewings were found in EBF and GE release plots. Low variations in ladybird abundance occurred before the end of wheat growing season. Our results contribute to promote the "push-pull" strategy in aphid biological control based on releaser use with GE and EBF acting as pest pushing and beneficial pulling stimulus with Z3H for aphid pulling.

Keywords: Wheat, Cereal aphids, infochemical, Natural enemy conservation

1. Introduction

Among aphid species, grain aphid [*Sitobion avenae* (Fabricius)], bird cherry-oat aphid [*Rhopalosiphum padi* (L.)], and rose-grain aphid [*Metopolophium dirhodum* (Walker)] are considered as the major pests that attack cereal crops by feeding on phloem and transmitting viruses (Van Emden and Harrington, 2007, Liu et al., 2009), particularly on winter wheat [*Triticum aestivum* L.(Poaceae)] in Europe (Poehling et al., 2007). Aphid populations often show strong year-to-year fluctuations (Kindlmann and Dixon, 2010) and are affected simultaneously by a range of biotic and abiotic factors (Leslie et al., 2009).

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 on herbivores and their natural enemies (Plepys et al., 2002, De Boer and Dicke, 2004, James and Price, 2004, James and Grasswitz, 2005, James, 2005, Yu et al., 2008, Lee, 2010, Snoeren et al., 2010, Wang et al., 2011). Several studies of the ecological importance of volatiles under natural conditions are performed to demonstrate their applicability in enhancing natural enemy abundance in strawberry (*Fragaria* × *ananassa* Duch.) (Lee, 2010), cotton (*Gossypium spp.*) (Yu et al., 2008), hops (*Humulus* *lupulus*) (James, 2005) and vineyard (James and Grasswitz, 2005), and reducing pest population in wheat (Prinsloo et al., 2007, Wang et al., 2011) and barley (Ninkovic et al., 2003).

The volatiles emanating from the herbivore-damaged plants may be produced in defense against herbivores but may also serve a secondary function in attracting the natural enemies of these herbivores (Turlings et al., 1990), and as recognition cues between two or more individuals (Howard and Blomquist, 2005).. Dicke et al. presented the first convincing evidence for an active release of volatiles by herbivore-infested plants that attract natural enemies of the herbivorous attackers (Dicke and Sabelis, 1987, Dicke et al., 1990). Aphid behaviour is also affected by density mechanism mediated by volatile compounds released at the feeding site when a certain density threshold is exceeded (Ninkovic et al., 2003). Further study revealed that these volatiles could increase the sensitivity of aphids to disturbance, and promote mobility of non-settled individuals (Pettersson et al., 1995).

Due to emanate from natural plants, essential oils suffer from fewer problems of animal and environmental toxicity compared with pesticides (Park et al., 2006, Prinsloo et al., 2007). Semiochemicals from plants should be considered as potential reliable infochemicals in relation to the efficacy to repell pests and attract natural enemies. Due to their long distance effect and easily way to produce and manipulate, these molecules are very good prospects for the use in crops by spraying or setting in slow release systems associated species to repel ovipositing insects from host plants and/or to guide them onto non-hosts (Pickett et al., 1991).

Japanese termite, (*Reticulitermes speratus*) (Park and Shin, 2005), sciarid fly [*Lycoriella ingénue* (Dufour)] (Park et al., 2006) and pine wood nematode (*Bursaphelenchus xylophilus*) (Park et al., 2005) were repelled by garlic (*Allium sativum*) extraction (GE) provide direct evidence that strongly aromatic crops such as garlic can provide an olfactory camouflage against insects which masks their normal host-finding or feeding cues (Perrin and Phillips, 1978). (E)-β-farnesene (EBF), an acyclic sesquiterpene olefin that occurs in a wide range of both plant and animal taxa, such as aphids (Francis et al., 2005) and peppermint (*Mentha x piperita*, L.) (Crock et al., 1997), was an effective kairomone for ladybird (Francis et al., 2004, Verheggen et al., 2007, Cui et al., 2012), lacewings (Zhu et al., 1999) and hoverflies (Almohamad et al., 2007). It has also been demonstrated to be the most common constituent of the aphid alarm pheromone (Edwards et al., 1973, Pickett and Griffiths, 1980, Wohlers, 1982, Yu et al., 2011, Vandermoten et al., 2012).

Herbivore- or wound-induced (Z)-3-hexenol (Z3H) can directly affect the physiology and behavior of herbivores (Wei and Kang, 2011). Z3H has been demonstrated to attract *Agrilus planipennis* in Laboratory and Field (Crook et al., 2009, Grant et al., 2010), and fruit moth, *Cydia molesta* (Dorn et al., 2003). Although it is difficult to conclude whether Z3H is an attractant or a repellent, accumulating evidences suggested that Z3H is, at least in part, important plant-derived infochemical that modulates the behavior of herbivorous insects, and the release of Z3H should be the defensive responses of the plants (Wei and Kang, 2011).

Extensive evidences imply that nearly all herbivorous insects and their natural enemies can perceive and positively respond to plant volatiles. In the present investigation, plant essential oil produced volatiles (EBF, GE and Z3H) were released in wheat crop. The aim of the study was to assess the potential of those infochemicals on aphid management strategy by reducing aphid abundance and promoting their natural enemies.

2. Materials and methods

Field experimental design

Field studies were conducted at the experimental fields of Gembloux Agro-Bio Tech, University de Liege, Namur Province of Belgium (50º33″N, 4º42″E) in 2011. The trial consisted of four treatments: (1) paraffin oil only in wheat crop (PO) as control, (2) (E)-β-farnesene release (EBF), (3) garlic extraction release (GE), (4) (Z)-3-hexenol release (Z3H). The releasers located on a yellow trap stick were set individually 20m apart the one from the other in a latin square dispositive with 3 replicates per treatment (12 releasers and 12 traps totally). Wheat (cv. Tybalt) was planted in 20-cm-apart rows at a rate of 350 seeds per m^2 on 18 February in 2011. No insecticides or herbicides were used in the whole experimental area.

Assessment of insect abundance and diversity

Yellow traps are frequently used to monitor insects in fields (Laubertie et al., 2006). Traps (26 cm diameter 10 cm depth) were attached to crabsticks and placed 10 cm above the surface of wheat plant. These traps were filled with water and a few drops detergent. One centimeter (in diameter) rubber septum was used to deposit solution of semiochemicals (formulated in paraffin oil for slow release action) and set on the trap stick, allowing the chemical to be released slowly. One hundred microliters of the solution were deposited in rubber septum every seven days. 76 micrograms of EBF was released from the formulation per seven days under the conditions of 20°C, relative humidity of 65% and air flow: 0.5 litre/min (Dr. S. Heuskin, unpublished data). Similar release was applied to other tested semiochemicals. The first application of chemical was made at the jointing stage on 4th of May, and subsequently applied every 7 days.

Traps were emptied and reset at 7-day intervals between 11th of May to 29th of June. Trap contents were decanted through a 1-mm mesh sieve and transferred to 70% ethanol in plastic 50-mL vials. In the laboratory, aphids and their natural enemies were sorted and identified to species, the abundance of each insect species was recorded.

To compare with aphid abundance in the traps, twenty wheat tillers were randomly selected each week and aphid density was visually assessed on selected tillers.

Statistical analysis

For all parametric tests, a data sqrt (n + 1) transformation was applied to stabilize the variance. The population densities of insects was compared among kind of infochemical releaser using a one-way analysis of variance (ANOVA) (SAS, 2001) followed by Fisher’s Least-Significant Difference s test (LSD).

3. Results

Abundance and diversity of aphids according to the kind of released infochemical

M. dirhodum and *S. avenae* were the predominant species on wheat, Z3H releasers were the most attracting aphid infochemical. Releasers of EBF and GE were found to significantly repell aphids within wheat field. Consistent tendency was observed when comparing the results from trapping and visual observation investigations. The total number of *M. dirhodum* was far higher than that of *S. avenae* both in observation and trap (Table 1 and Figure1). In addition, several wheat non-target aphid species were recorded in traps: *Cavariella aegopodii* (scopoli)*, Aphis fabae* Scopoli*, Macrosiphum euphorbiae* (Thomas)*, Myzus persicae* Sultzer*, Rhopalosiphum maidis* (Fitch)*, Cavariella ihedbaldi, Nasonovia ribisnigri* (Mosley)*, Phyllaphis fagi* L*, Chaitophorus spp and Capitophorus spp.*

According to visual observation and trapping investigations, the population dynamics of *M. dirhodum* and *S. avenae* in each treatments exhibited the same trend as wheat growing, and the population densities of *M. dirhodum* and *S. avenae* reached their occurrence peaks on June 15th, and June 22nd, respectively (Figure 2). For visual observation within field, Z3H attracted mainly *M. dirhodum* both in peak period and ton whole observation period while EBF and GE were found to repell aphids (Peak: $F_{3,\,8}$ = 18.95, P<0.01; Total: $F_{3,\,8}$ = 34.45, P<0.01). Similarly, significant differences for *S. avenae* were detected with lower abundance with EBF and GE releasers (Peak: $F_{3,\,8}$ = 89.30, P<0.01; Total: $F_{3,\,8}$ = 45.55, P<0.01).

Consistent with the results of visual observations, the abundance of *M. dirhodum* in traps was higher with Z3H and lower with EBF and GE releasers both in the aphid occurrence peak and on total captures (Peak: $F_{3,8}$ = 56.30, P<0.01; Total: $F_{3,8}$ = 86.27, P<0.01). The highest abundance of *S. avenae* was found in traps with Z3H. EBF and GE releasers were found to also repell *S. avenae* both in the occurrence peak period and total experimental duration (Peak: $F_{3,8}$ = 56.30, P<0.01; Total: $F_{3,8}$ = 86.27, P<0.01). Comparing the data obtained from visual observations and trapping, the consistency of results for *M. dirhodum* and *S. avenae* with identical infochemicals was confirmed.

Abundance and diversity of aphid natural enemies according to the kind of released infochemical

Main natural enemies of cereal aphids were lacewings, the most abundant aphid predator group (47.8%), secondly hoverflies (39.4%) and ladybirds (12.8%). Focusing on predatory species, *E. balteatus*, *C. carnea* and *H. axyridis* were the predominant species on wheat. For the total numbers of aphidophagous species, the proportion was higher for EBF, GE and Z3H than PO as control (Table 1). Not all the collected hoverflies were aphidophagous species (*Eristalis pertinax, Helophilus trivitatus, Cheilosia spp, Eristalis tenax, Eristalis arbustorum*). We focused on aphid predator and their diversity was presented in Table 1.

The hoverfly population density had a peak from 22nd to 29th of June (Figure 3A). There was no significant difference in hoverfly population density among tested infochemical releasers before peak occurrence period. After, the hoverfly density related to EBF releases was significantly higher than that related to Z3H ($F_{3,8} = 4.46$, $P<0.05$). No significant difference in total hoverfly abundance among treatments was detected ($F_{3,8} = 1.64$, $P=0.26$).

Lacewings reached its occurrence peak in all treatments on June 15th along with the peak of *M. dirhodum* (Figure 3B). The population density of lacewings in each treatment was low before June 8th. Aphid density in GE released plots was significantly higher at the occurrence peak period ($F_{3,8} = 3.03$, $P<0.05$). No significant difference in total lacewing abundance among treatments was detected ($F_{3,8} = 1.25$, $P=0.36$).

Finally, no significant variation in ladybird population dynamic for each treatment was observed before June 22nd. Moreover, ladybirds reached their occurrence peak in all treatments at the end of wheat growing when aphids population declined rapidly in field (Figure 3C). No significant difference in abundance of ladybirds among treatments was detected both in peak period and total experimental duration (Peak: $F_{3,8}$ = 1.92, P=0.21; Total: $F_{3,8}$ = 0.52, P=0.68).

4. Discussion

The population densities of cereal aphids and their natural enemies in wheat were significantly influenced by the tested infochemical releasers, mainly with EBF and GE. This supports the viewpoint that these volatiles play a significant role in behavioural ecology of aphids, and demonstrates their potential for use in pest control. Several reasons reviewed by Kunert et al. (2010) could contribute to understanding of low abundance of *M. dirhodum* and *S. avenae* in EBF released plots. Firstly, EBF emission may directly prevent aphid settling. This has been reported for the wild potato (*Solanum berthaultii*) which repels the green peach aphid (*Myzus persicae*) by the release of EBF (Gibson and Pickett, 1983). Secondly, EBF might also reduce aphid growth rate by disrupting feeding (Pickett et al., 1992). Thirdly, EBF-induced wing formation and might reduce aphid population size. Since winged offspring leave their host plant before starting reproduction, plants which produce EBF could reduce their aphid load (Kunert et al., 2005, Hatano et al., 2010). Under natural conditions, plants emit chemical signals in response to attack by insect herbivores that recruit the herbivores' natural enemies (Verheggen et al., 2010). Then, it is possible that EBF released in plots mainly improve the efficiency of the natural enemies on the host location step. This hypothesis is supported by the results of our study that the population densities of hoverflies were higher in EBF released treatment at the peak periods. Nevertheless, there were some exceptions for the influence of EBF on lacewings and ladybirds in our investigation. The amount of infochemical in releasers may determine the probability of predator response. Shiojiri et al. (2010) showed that seedlings of a cabbage variety attracted more parasitoids (*Cotesia glomerata*) when there were more herbivores on the plant. The further study should be performed to demonstrate the phenomenon.

Aphids’ perception of volatile cues is adapted for avoidance of non-host plants (Pickett and Glinwood, 2007). Garlic plants represent non-hosts to cereal aphids, and its extraction is likely to be unsuitable for feeding by aphids. The significantly lower population densities of *M. dirhodum* and *S. avenae* were found in GE released plots than that in PO released plots. It was worth noting that GE exhibited the attractive effect for lacewings when comparing with PO released in plots. Moreover, there was no negative influence of GE on hoverflies and ladybirds in field. To the best of our knowledge, no more published studies have shown a signaling function for GE in helping plants to recruit natural enemies.

Based on the current available knowledge, wound-induced ubiquitous

(Z)-3-hexenol, a C6-alcohol synthesized in the lipoxygenase/HPL pathway, was also proved to be the most important infochemical for the herbivore repellence/attraction in tritrophic interactions (Wei and Kang, 2011). Volatiles from wheat and oat seedlings elicited attraction in apteral and alatae *Rhopalosiphum padi*. Cereal volatiles were identified by GC-MS and olfactometer tests were performed with each compound, showing attraction of aphids was elicited by (E)-2-hexenyl acetate, (Z)-3-hexenol, (Z)-2-hexenol and so on (Quiroz and Niemeyer, 1998). In accordance with Quiroz's results, the highest population densities of *M. dirhodum* and *S. avenae* were found in Z3H released treatment (Figure.2).

Those volatiles in our study showed the aphid repellence or attraction and the natural enemy attraction or no influence, promoting the "push-pull" strategy in aphid biological control that Z3H could be regard as the pull stimulus and the push stimulus were GE and EBF. Recent studies have provided evidence for the potential use of synthetic volatiles as aids to enhancing conservation biological control in crop ecosystems (Sabelis et al., 1999, James, 2003, James and Price, 2004). Targeting the right volatiles for enhanced emission should lead to ecologically and economically sound ways of combating important pests. However, a remaining question surrounding the use of these materials in integrated pest management is to what are the ecological consequences of providing synthetic volatiles to predators and parasitoids in the absence of their prey.

Therefore more detailed work on its ecological consequences, application rate, dose and duration under field conditions need to be done before those volatiles can be used to develop novel insect pest control strategies.

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Tables and Figures

Table

Table 1. Total numbers of aphids and their natural enemies recorded in yellow traps in different crop systems throughout 2011 growing season

Species	Treatments				
	paraffin oil	(E)-β-farnesene	garlic extraction	(Z)-3-hexenol	%[a]
Aphids					
Metopolophum dirhodum (Walker)	896	585	582	1122	89.5
Sitobion avenae (Fabricius)	138	35	54	148	10.5
Diversity and abundance of aphid species%	29.0	17.4	17.9	35.7	
Ladybirds	12.8%[b]				
Harmonia axyridis Pallas	18	21	22	28	66.8
Coccinella septempunctata L.	9	9	8	3	21.8
Propylea 14-punctata	3	3	1	0	5.3
Harmonia 4-punctata	2	1	0	0	2.3
calvia 14-guttata	2	0	0	0	1.5
Hippodamia Variegata (Goeze)	1	1	0	1	2.3
Hoverflies	39.4%[b]				
***Episyrphus balteatus* De Geer**	69	108	85	76	82.6
***Scaeva pyrastri* L.**	2	0	0	7	2.2
***Sphaerophoria scripta* L.**	12	16	9	8	11.0
Melanostoma scalare	0	3	0	1	1.0
Metasyrphus corollae	5	1	2	5	3.2
Lacewings	47.8%[b]				
Chrysoperla carnea Stephens	95	128	152	121	100.0
Total numbers of aphidophagous species	218	291	279	250	

Proportion of total numbers of aphidophagous species%	21.0	28.0	26.9	24.1

[a] Relative abundance of each species by family

[b] Relative occurrence of each family in aphidophagous guild

Figures

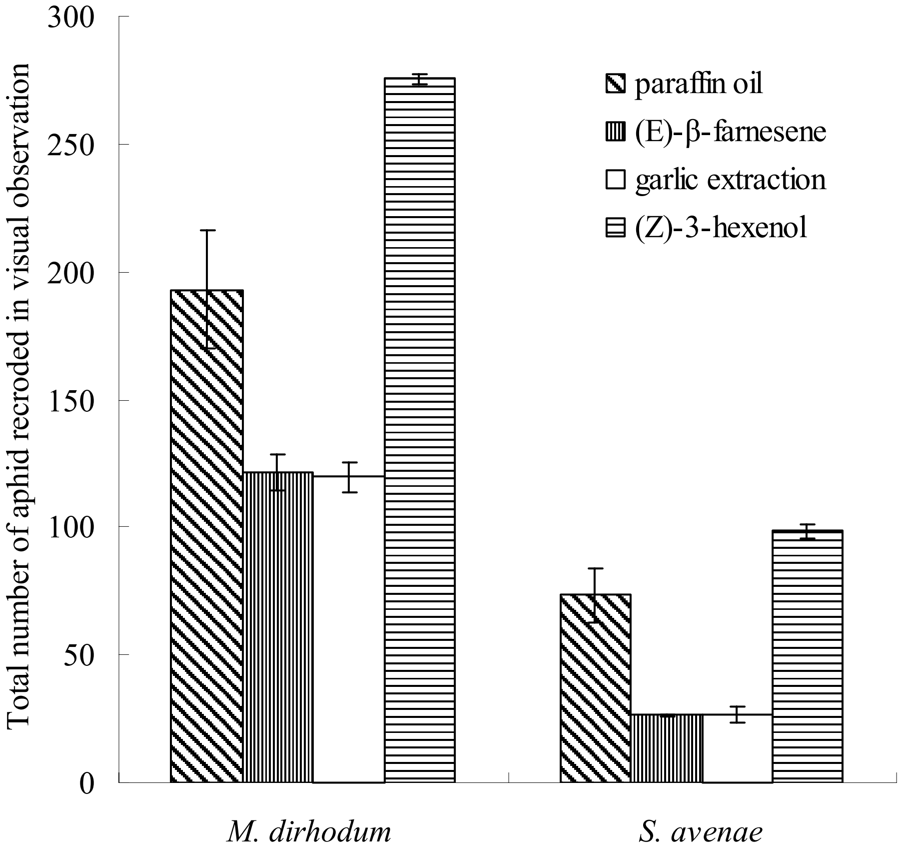
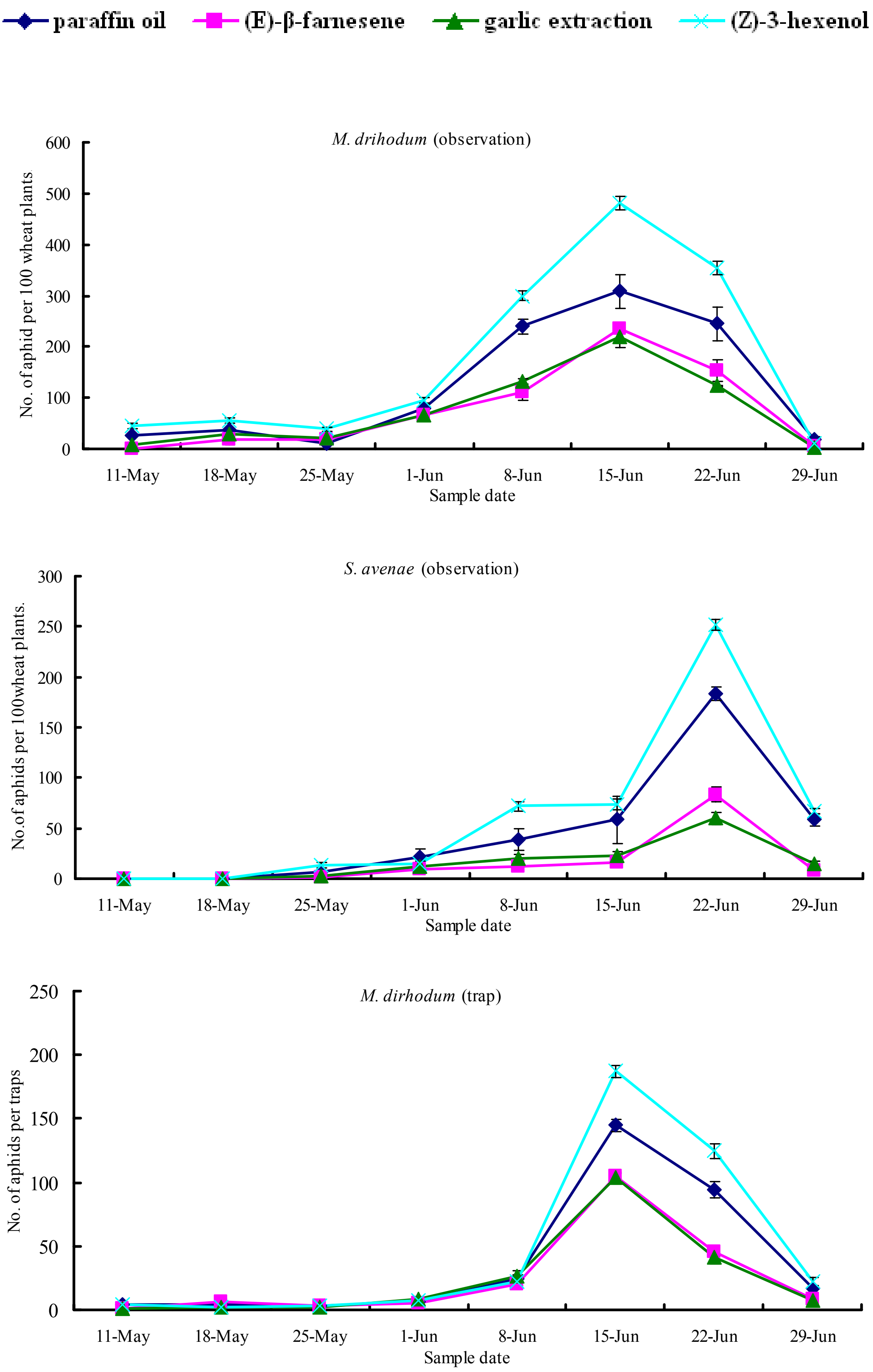


Figure. 1 Total number of aphid (Mean±SEM) recorded in visual observation according to the kind of released infochemical


paraffin oil
(E)-β-farnesene
garlic extraction
(Z)-3-hexenol
M. drihodum (observation)
No. of aphid per 100 wheat plants
0
100
200
300
400
500
600
11-May
18-May
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
Sample date
S. avenae (observation)
No.of aphids per 100wheat plants.
0
50
100
150
200
250
300
11-May
18-May
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
Sample date
M. dirhodum (trap)
No. of aphids per traps
0
50
100
150
200
250
11-May
18-May
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
Sample date

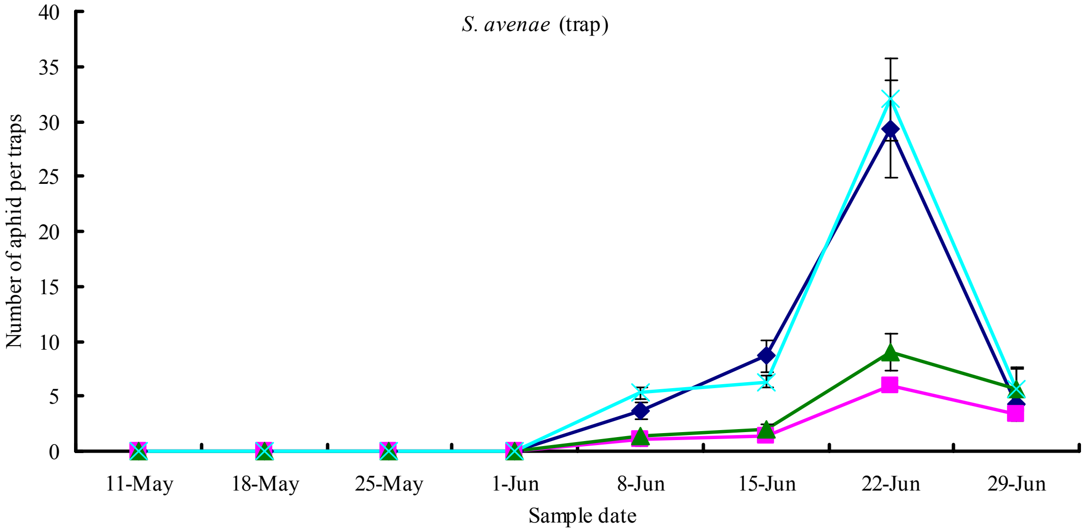


Figure 2. Seasonal abundance of aphids (Mean±SEM) recorded in the different treatments investigated in the 2011.

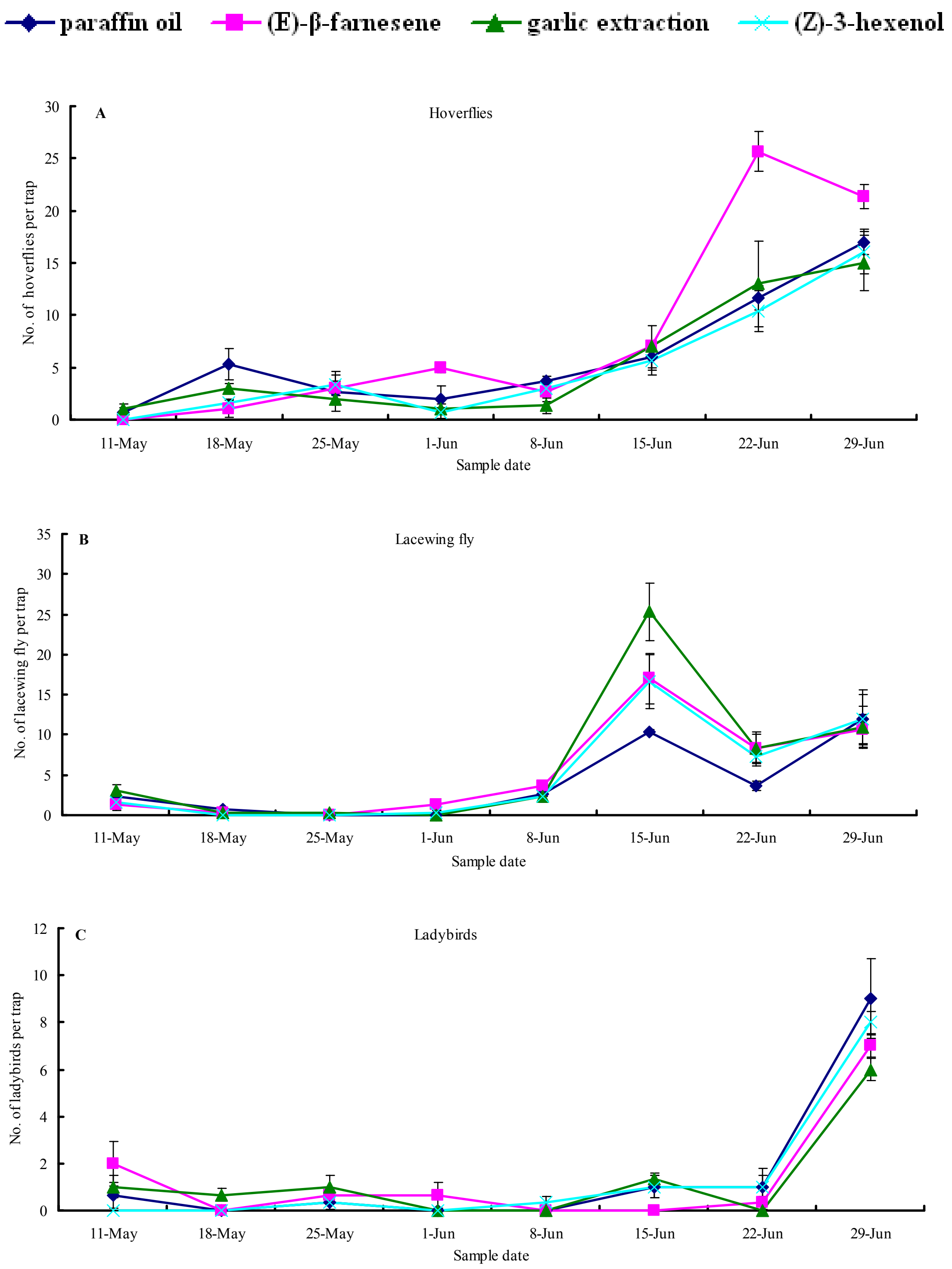


Figure 3. Seasonal abundance of natural enemies (Mean±SEM) recorded in the different treatments investigated in the 2011.

V. 2. The influence of garlic intercropping or active emitted volatiles in releasers on aphid and related beneficial in wheat fields in China

Haibo Zhou[a,b], Julian Chen[a], Frédéric Francis[b], Yong Liu[c], Eric Haubruge[b], Claude Bragard[d], Jingrui Sun[a]

[a] State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China,

[b] Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux, 5030, Belgium,

[c] College of Plant Protection, Shandong Agricultural University, Taian, 271018, PR China,

[d] Université catholique de Louvain, Earth & Life Institute, Applied Microbiology-Phytopathology, Louvain-la-Neuve, 1348, Belgium

Reference-Haibo Zhou, Julian Chen, Dengfa Cheng, Yong Liu, Claude Bragard, Eric Haubruge, Frédéric Francis (2012). The influence of garlic intercropping or active emitted volatiles in releasers on aphid and related beneficial in wheat fields in China. Under review in *Journal of Integrative Agriculture*

Abstract

In order to develop biological control of aphids by a "push-pull" approach, intercropping using repellent emitting plants were developed in different crop and associated plant models. Garlic is one of the potential plant that could be inserted in crops to decrease the pest occurrence in neighboring crop plots. In this study, field works were conducted in wheat fields in Langfang experimental station, in China from October 2009 to July, 2010 during wheat developmental season. The effect of wheat intercropping with garlic but also the volatiles emission on the incidence of the wheat aphid, *Sitobion avenae* Fabricius (Homoptera: Aphididae) was assessed. Natural beneficial occurrence and global yields in two winter wheat varieties that were susceptible or resistant to cereal aphid were also determined comparing to control aphid plots without garlic plant intercrop nor semiochemical releaser use in the fields. *S. avenae* was found to be lower in garlic oil blend treatment (GOB), diallyl disulfide treatment (DD) and wheat-garlic intercropping treatment (WGI) when compared to the control wheat plots for both two varieties ($P<0.01$). Both intercropping and application of volatile chemicals emitted by garlic could improve the population densities of natural enemies of cereal aphid, including ladybeetles and mummified aphids. Ladybeetle population density in WGI, GOB and mummified aphids densities in WGI, DD were significantly higher than those in control fields significantly for both two varieties ($P < 0.05$). There were significant interactions between cultivars and treatments to the population densities of *S. avenae*. The thousand grain weight and yield of wheat were also increased compared to control. Due to their potential alternatives as a biological control agent against wheat aphid, garlic intercropping and related emitted volatiles are expected to contribute to the further improvement of integrated pest management systems and to potentially reduce the amount of traditional synthetic pesticides applied in wheat fields.

Key words:

wheat, garlic, intercropping, semiochemical release, *Sitobion avenae*, natural enemies

1. Introduction

Wheat, *Triticum aestivum* L., and garlic, *Allium sativum* L., are important crops for the people of the world as well as China. The English green aphid, *Sitobion avenae* Fabricius (Homoptera: Aphididae), is a ubiquitous pest that attacks wheat throughout its growth stages in north China (Cai et al., 2004; Zhao et al., 2009). One approach to control of this pest is to develop management systems using diversified agroecosystems. Intercropping, 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 (Hassanali et al., 2008; Konar et al., 2010; Smith & McSorley, 2000; Suresh et al., 2010; Vaiyapuri et al., 2010). For example, from 2002 to 2004, Ma et al examined Strip cropping of wheat and alfalfa, *Medicago sativa,* for its utility to improve the effectiveness of biological control of the wheat aphid, *Macrosiphum avenae* by the mite, *Allothrombium ovatum* (Ma et al., 2007). Wheat-garlic intercropping, planting row in an 8:3 ratio, can reduce the population of *S. avenae* by promoting natural enemies in wheat fields experiments (Wang et al., 2008). The benefits of intercropping for controlling aphids and encouraging their natural enemies have also been studied in: wheat and oilseed rape, *Brassica napus* L. (Wang et al., 2009); cowpea, *Vigna unguiculata* (L.) Walp and sorghum, *Sorghum bicolor* (L.) Moench (Hassan, 2009), wheat and Pea *Pisum sativum* Linn (Zhou et al., 2009a; Zhou et al., 2009b). Intercropping has also been described potentially increasing crop yields by suppressing pest outbreaks (Mucheru-Muna et al., 2010; Rao et al., 2010; Sarker et al., 2007; Vaiyapuri & Amanullah, 2010; Zhang et al., 2007). In addition, it is important to take the resistant levels to aphids of a host plant into consideration. In an intercropping system, wheat varieties that are susceptible or moderately resistant to wheat aphids may reduce cotton aphids more effectively than an aphid-resistant variety by enhancing predators to suppress cotton aphids during the cotton seedling stage (Ma et al., 2006).

However, volatiles produced by non-host plants often affect the behavior of aphids and their natural enemies, these may vary genetically among plants. Intercropping with the non-host molasses grass, *Melinis minutiflora*, significantly decreased levels of infestation by stem-borers in the main crop and also increased larval parasitism of stemborers by *Cotesia sesamiae*. Volatile agents produced by *M. minutiflora* repelled female stem-borers and attracted foraging female *C. sesamiae* (Khan et al., 1997). Due to the inherent variability, an important modification of this method is the external application of volatile semiochemicals in the field, which have a stabilizing effect and may reduce populations of the aphids *Diuraphis noxia* (Prinsloo et al., 2007), and *Rhopalosiphum padi* (Glinwood & Pettersson, 2000; Ninkovic et al., 2003). Essential oils, obtained by steam distillation of plant foliage, and even the foliage itself of certain aromatic plants have traditionally been used to protect stored grain and legumes, and to repel flying insects (Isman, 2000). Diallyl disulfide, an essential component of garlic volatiles (Edris & Fadel, 2002), and in a fumigation bioassay, had insecticidal activity on the larvae of Japanese termite, *Reticulitermes speratus* (Park & Shin, 2005) and mushroom fly, *Lycoriella ingenua* (Park et al., 2006). However, few studies have investigated the effects of garlic oil blend and its components on *S. avenae* control in wheat fields.

The primary objectives of this study were thus to compare the effects of wheat monoculture, wheat-garlic intercropping (wheat cultivars with different resistant levels to wheat aphids), treatment with a garlic oil blend, and diallyl disulfide release in wheat fields on *S. avenae*, their natural enemies, and overall crop yield. It could provide a potential strategy that can contribute to the biological control to reduce the aphid infestations.

2. Materials and methods

Wheat and garlic varieties

Two wheat varieties, *Triticum aestivum,* with different levels of resistance to *S. avenae* were provided by the Institute of Plant Protection at the Chinese Academy of Agricultural Science in Beijing: cv. Beijing837 (susceptible) and cv. Zhengzhou831 (resistant). The garlic variety, *Allium sativum* L. cv. Zhongnong4 was also used in this study. This variety is currently used commercially in Huang-Huai-Hai plain, China.

Chemicals

Diallyl disulfide (purity 80%, remainder mainly allyl sulfides) and Garlic oil blend (30-50 wt. % Diallyl disulfide, 10-13 wt. % Diallyl trisulfide, 5-13 wt. % Allyl disulfide) were purchased from Sigma-Aldrich, Inc (Missouri, US).

Field experimental design

Field experiments were conducted at the Langfang Experimental Station of the Plant Protection Institute, CAAS, Hebei Province of China (39°30′N, 116°37′E) in 2010. Wheat and garlic were planted with 20 and 40 cm distance between rows in wheat and garlic, respectively.

A conventional randomized block design was used, with treatment plots (10×8m) randomly repeated in each of four blocks. The following treatments were compared: (a) WGI, wheat-garlic intercropping by planting row in 8:3 ratio; (b) GOB, the release of Garlic oil blend in wheat field; (c) DD: the release of diallyl disulfide in wheat field; (d) CK: control, wheat monoculture without garlic plant intercrop nor semiochemical releaser use in the fields. A two-meter wide area was set around the plots to decrease potential border effects on insect dispersion. No pesticides or herbicides were applied on the fields during the entire growth stage of wheat and garlic.

Release of chemicals in fields

A rubber tube (10cm×0.05cm diameter) as the releaser loaded with 10μl candidate volatile substances was hung in wheat fields at a height of 1.2 m, and five releasers were used in each single plot. The first introduction of releasers was made on 22 April 22 (at the setting stage of wheat), and chemicals were subsequently supplied every 7 days until aphid counting ended.

Sampling of insects

Due to aphid parasitoids being difficult to count in the field, the number of mummified aphids found was examined. Mummified aphids and aphid densities on plants were counted and recorded in each plot in five "one square meter plot". Within each sampling plot, thirty randomly selected wheat tillers were used as one sampling unit. Lady beetles on all wheat plants within the "one square meters plot", and covering three rows of wheat were counted in the center of each plot. Aphids was sampled in wheat every 4-days from April 24th to June 7th. Ladybeetles and mummified aphids were sampled every 4-days from May 16th to June 5th.

Crop yields

Yields and thousand grain weights of wheat were assessed by harvesting and weighing crop products from each plot. This resulted in the calculation of yields in kg .ha^{-1}. Thousand grain weight was evaluated by weighing two samples of 500 kernels for each plot.

Statistical analysis

All data of insect population densities related to the different treatments in field were analyzed using one-way analysis of variance (ANOVA) (SAS, 2001) followed by Least-Significant Difference test (LSD). The effects of varieties and treatments on aphids and their natural enemies were analyzed using the General Linear Model (GLM) procedure. The data used in ANOVA and GLM were transformed by square root, when necessary, to meet assumptions of normality before variance analysis. A probability level of <0.05 was considered statistically significant.

3. Results

Aphid population density

S. avenae population densities differed significantly among the four treatments at sampling date in Beijing 831 and Zhengzhou 837 (Figure 1). *S. avenae* populations decreased dramatically from late May to early June, and peak numbers were found in late season sampling in both cultivars examined.

Aphid population reached its peak in the two cultivars on May 22. However, during this peak period, aphid population density with the control treatment was significantly higher than that seen in any other treatments in both cultivars (Beijing 837: $F_{3,12}$ = 111.62, P <0.01; Zhengzhou831: $F_{3,12}$ = 215.41, P <0.01). The highest abundance of aphids was observed in the CK treatment, and lowest in DD and GOB treatment with both Beijing 837 and Zhengzhou 831.

Ladybeetle population density

There were three species of ladybeetles, *Coccinella septempunctata* L., *Harmonia axyridis* Pallas and *Propylaea japonica* Thunber, found in wheat fields during the sampling period. The ladybeetle populations (all species) of each block for two cultivars (Beijing 837 and Zhengzhou 831) are shown in Figure 2. The ladybeetle populations in WGI, DD and GOB blocks were significantly higher compared to the CK blocks for both cultivars at the peak dates with the exception of DD in Beijing 837 (Beijing 837: $F_{3,12} = 52.34$, $P < 0.01$; Zhengzhou831: $F_{3,12} = 131.46$, $P < 0.01$). And WGI had the highest number of ladybeetles, followed by GOB and DD.

Mummified aphids density

The peak Mummified aphid densities was occurred in all treatments on May 30 (Figure 3). On this date, Mummified aphids densities were lower in CK blocks than in DD and WGI blocks (Beijing 837: $F_{3,12} = 20.41$, $P < 0.05$; Zhengzhou831: $F_{3,12} = 21.32$, $P < 0.01$). Although GOB also increased parasitism of *S. avenae*, there was no significant difference compared to CK treatments in the two cultivars.

Two-factor effects

A summary of the statistical analyses on the effects of treatments on the mean number of *S. avenae*, ladybeetles, and Mummified aphids are given in Table 1. There was a significant difference seen *S. avenae* ($P < 0.0001$) between different treatments. Wheat cultivars also influenced observed numbers of S. *avenae* ($P < 0.0001$) and ladybeetles ($P < 0.0001$). There were significant interactions between cultivars and treatments for *S. avenae* ($P = 0.0475$). However there was no significant difference detected in mummified aphids in the wheat cultivars and treatments and their interactions.

Yields

Wheat thousand grain weights and yield were both increased when compared with CK, and significant differences were detected in all pairwise comparisons between WGI, DD, GOI and CK except with DD in thousand grain weight of Zhengzhou831. The highest thousand grain weight and yield were observed with the DD treatment, but there were no significant difference among WGI, DD and GOB except in yield of Zhengzhou831, data was shown in Tab. 2.

4. Discussion

Increasing agrobiodiversity by crop intercropping and application of plant essential oils in fields may provide potential alternatives to those currently used to control *S. avenae.* Alternative treatments can avoid environmental pollution and health problems caused by the extensive use of traditional synthetic pesticides.

Intercropping is regarded as one approach to pest control in resource-poor regions, as it exploits the ‘push–pull’ strategy, whereby insects are repelled from a harvestable crop and simultaneously attracted to a ‘discard’ or ‘trap’ crop (Khan et al., 1997). The push-pull strategy is a useful tool for integrating pest management programs, reducing pesticide input (Cook et al., 2007), and maximizing the efficacy of behavior manipulating stimuli through the additive and synergistic effects of a non-host crop. Our results indicated that the abundance of *S. avenae* was lower, with both varieties, the wheat-garlic intercropping system than in wheat monoculture. This may be due to two factors: ① garlic is a stimulus for push components to make wheat resources hard to locate, unattractive, or unsuitable to aphids; and/or ② intercropping systems that increase crop diversity in the agroecosystem significantly preserved and augmented more ladybeetles and mummified aphids than monoculture wheat fields. Similar phenomenon was also observed in wheat-garlic (Wang et al., 2008), wheat-alfalfa (Ma et al., 2007) and maize-sorghum (Khan et al., 1997) intercropping system. The results of this study further demonstrated the effects of intercropping on aphids and their natural enemies. Wheat varieties (Beijing837) that are susceptible to wheat aphid might reduce wheat aphids more effectively than an aphid-resistant variety (Zhengzhou831) in the intercropping system. This may occur by an attracting more ladybeetles to suppress wheat aphids. There was no significant difference on mummified aphids densities between the varieties.

Aphid behavior is affected by volatile compounds which can increase the sensitivity of aphids to disturbance, and promote mobility of non-settled individuals (Pettersson et al., 1995), such as methyl salicylate (Ninkovic et al., 2003). Some volatile compounds (e.g. *cis*-Jasmone) also may induce plant defenses and reduce *S. avenae* populations in the field test (Bruce et al., 2003). Lower densities of aphids and increase in their natural enemies were found in fields with applications of semiochemicals from garlic. Although no significant difference in mummified aphids densities were seen between fields with garlic oil blend and control was measured, ladybeetle population density in fields with garlic oil blend and mummified aphids densities in fields with diallyl disulfide were significantly higher than those in control fields. A significant effect of semiochemicals treatment on aphids and their natural enemies was observed between susceptible (Beijing 837) and resistant(Zhengzhou 831) varieties, possibly due to complex interactions between the chemical, plant variety and growing environment (Prinsloo et al., 2007). Thousand grain weight and yield of wheat were also increased in treatment fields, except for the thousand grain weight in the GOB field for Zhengzhou831, and there were no differences among WGI, GOB and DD except in yield of DD treatments for Zhengzhou831.

Aphid perception of volatile cues is adapted for avoidance of non-host plants, and they can detect a wide range of chemical compounds (Pickett & Glinwood, 2007; Prinsloo et al., 2007). Garlic oil blend and diallyl disulfide are components of the essential oils of garlic, *A. sativum*, which represent non-hosts to wheat aphids, and are likely to be unsuitable for their orientation. Intercropping with garlic can also be used as an approach to control wheat aphids by conserving and enhancing populations of their natural enemies, and consequently also reducing the chemical dependency in wheat agroecosystems. Further research needs to be done to evaluate the mechanisms of how garlic and its volatiles affect the natural enemies in a complex agroecosystem.

5. Acknowledgments

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Figures and Tables

Figures

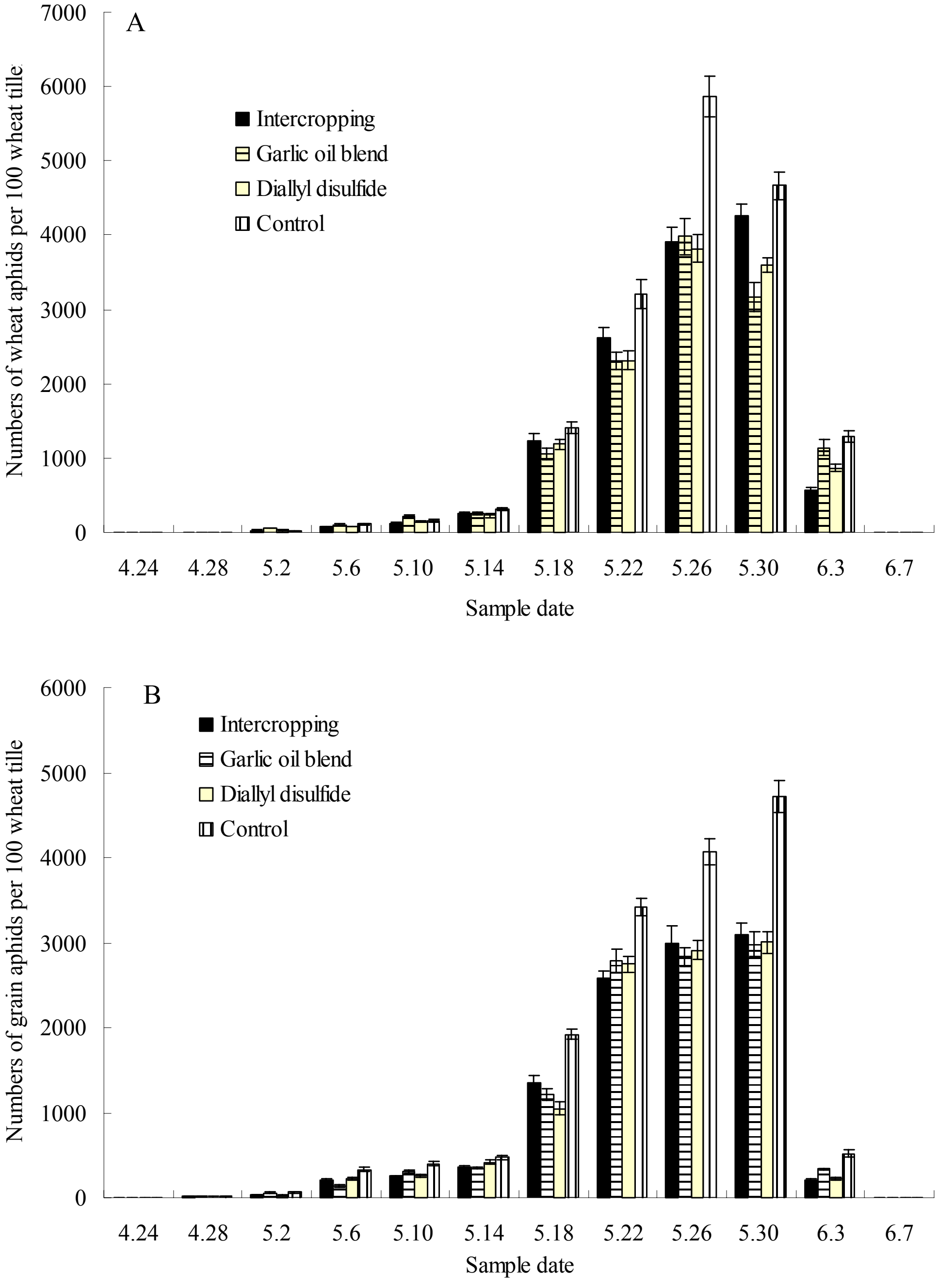

Figure 1 Population dynamics of *S. avenae* (Mean ±SE) in different treatments. (A: Beijing 837, B: Zhengzhou 831).

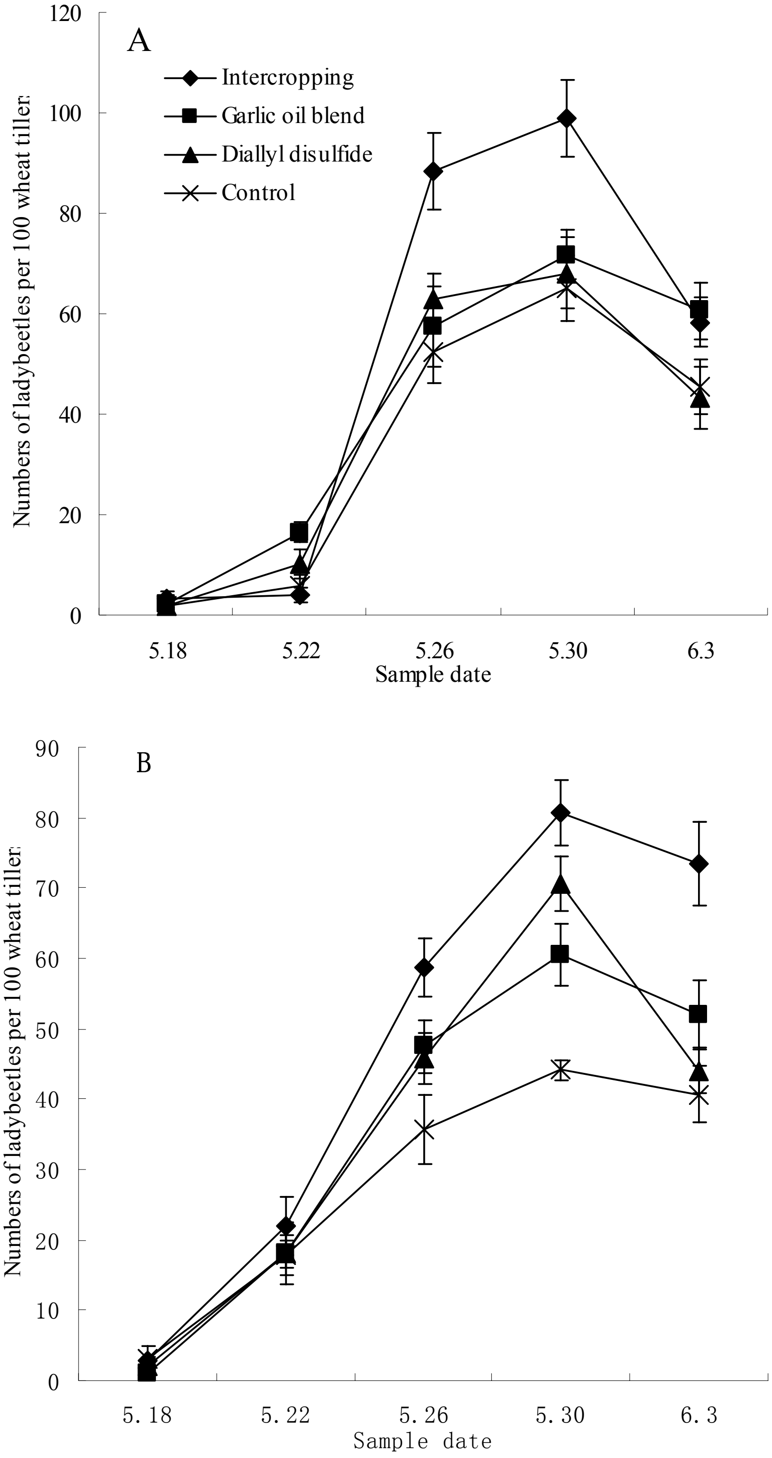

Figure 2 Population dynamics of ladybeetles (mean±SE) in different treatments. (A: Beijing 837, B: Zhengzhou 831).

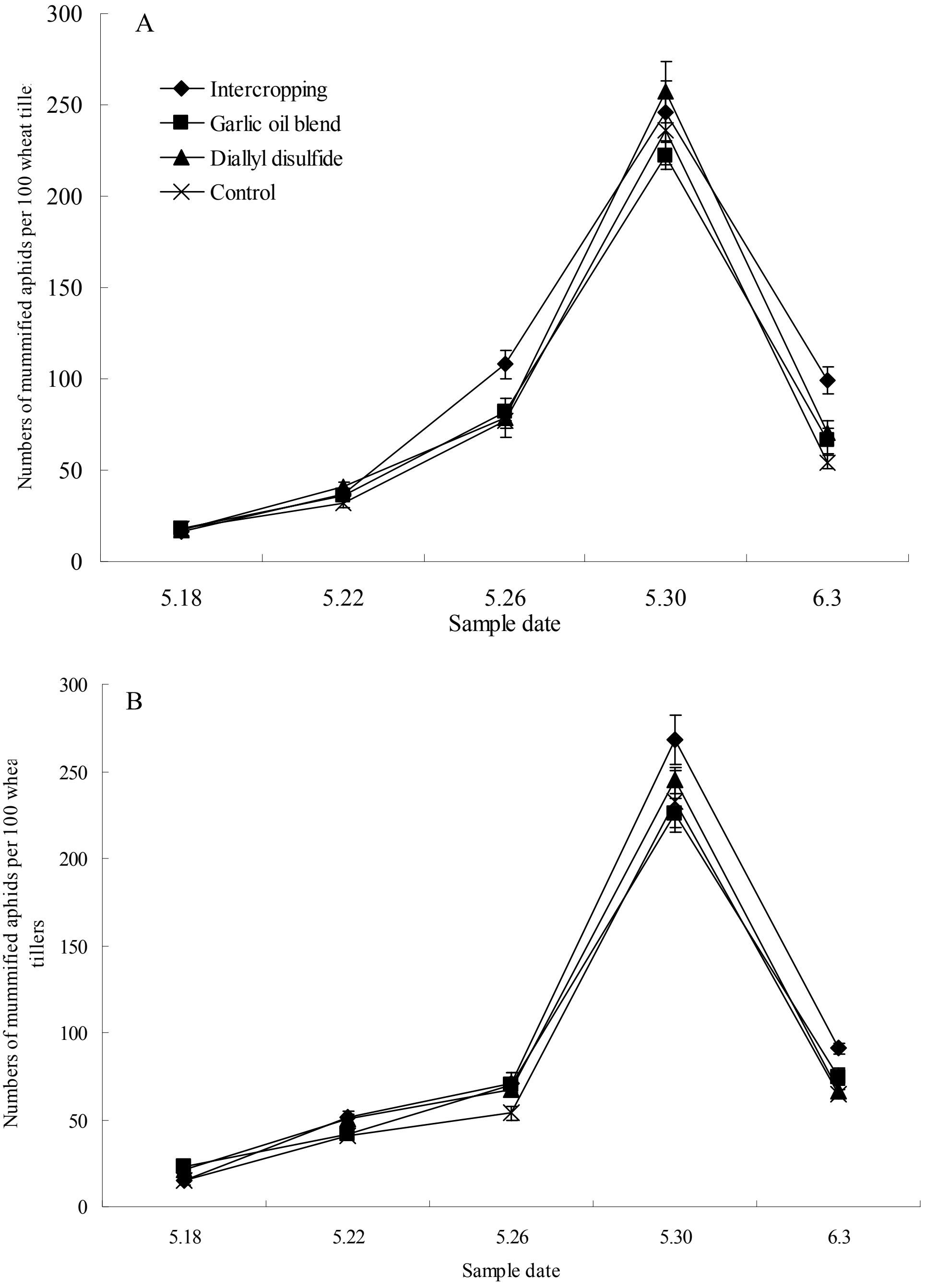

Figure 3 Population dynamics (mean±SE) of mummified aphids treatments. (A: Beijing 837, B: Zhengzhou 831).

Tables

Tab.1 F-test on effects of wheat cultivars and treatments on the abundance of S. *avenae* and natural enemies in wheat field

Source of variation	d.f.	S. *avenae*		Ladybeetles		Mummified aphids	
		F	P	F	P	F	P
Wheat variety	1	155.52	<0.0001	24.58	<0.0001	1.24	0.2786
Treatment	3	370.47	<0.0001	0.18	0.9071	1.04	0.3943
Wheat variety*Treatment	3	3.13	0.0475	0.66	0.5857	0.72	0.5521

Tab.2 Thousand grain weight and yield in different treatments

Treatment	**Thousand grain weight(g)**		**Yield(kg/Ha)**	
	Beijing837	**Zhengzhou831**	**Beijing837**	**Zhengzhou831**
Intercropping	35.29±2.17a	36.62±1.39a	4771.2±228.5a	5726.4±204.0b
Garlic oil blend	35.60±1.44a	36.40±2.24ab	4737.6±153.9a	5736.0± 94.5b
Diallyl disulfide	37.38±0.98a	39.18±2.79a	5020.8±152.4a	6182.4±228.8a
Control	32.53±1.03b	33.38±1.49b	4075.2±269.4b	4814.4±115.6c

Mean values ±SE in the same column followed by different letters are significantly different (ANOVA, LSD test, differences considered significant at $P < 0.05$).

Chapter VI: *THE EFFECT OF WHEAT-PEA INTERCROPPING ON CEREAL APHIDS AND THEIR NATURAL ENEMIES*

General Introduction to Chapter VI

semiochemicals from non-plants are so powerful in their effects and are so easily manipulated, that there are good prospects for the use of plant extracts as sprays on crops or associated species to repel ovipositing insects from host plants and/or to guide them onto non-hosts. Non-host plant could also provides an olfactory camouflage against insects which masks their normal host-finding or feeding cues. Therefore, intercropping with non-host plant in wheat fields should be taken into account in aphid control.

Intercropping, the agronomic practice for the development of sustainable food production systems and effective ways for increasing biodiversity, plays an important role in controlling pests and protecting beneficial insects relevant to enhancing biodiversity in an agroecosystem. One important solution could be to diversify agroecosystems by increasing the number of species grown and using more leguminous crops. Also, legume intercrops are also potential sources of plant nutrients that complement/supplement inorganic fertilizers by direct nitrogen transfer from the legume to cereal. Establishing flowering plants in and around fields to provide pollen and nectar resources for natural enemies has shown promise as a strategy to enhance biological control of crop pests.

VI.1. Beneficial effect of associating pea to wheat: a laboratory approach on aphid and related predator behavious

Haibo Zhou[a,b], Julian Chen[b], Yong Liu[c], Claude Bragard[d], Eric Haubruge[a], Frédéric Francis[a]

a Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux, 5030, Belgium

b State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China

c College of Plant Protection, Shandong Agricultural University, Taian, 271018, PR China

d Applied Microbiology-Phytopathology, Earth and Life Institute, Université catholique de Louvain, Louvain-la-Neuve,1348, Belgium

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ABSTRACT

Plant diversification and agro-ecosystem management could provide a potential strategy for pest control by influencing herbivore distributions both directly by mediating host-plant selection and indirectly by modifying the behaviour of natural enemies. Assessment of associating healthy but also aphid infested pea and wheat plants in several combinations, on *Harmonia axyridis*, *Episyrphus balteatus* beneficials as well as *Sitobion avenae* aphid was carried out in the laboratory by developing behavioural assays. The frequencies of searching and oviposition parameters of hoverfly were influenced by the selected combinations. In addition, the oviposition frequency of *E. balteatus* was improved when related to the presence of pea in wheat plants. Dual choice tests using a two way olfactormeter revealed that odors from combinations of wheat and pea had limited effect on the preference of *H. axyridis*. Healthy wheat plants were preferred by *S. avenae* to empty control. Also, the presence of conspecific on wheat proposed plant did not provide any more attraction to *S. avenae* alate. The presence of *Acyrthosiphon pisum* infested pea induced a significant repellent effect on *S. avenae*. These results were discussed to promote intercropping and aphid control in further field experiments including the effect on beneficials in a push-pull approach by attracting the beneficial and repelling aphid pests.

Key words: wheat, pea, *Sitobion avenae*, *Harmonia axyridis*, *Episyrphus balteatus*, behavioural observation

1. Introduction

To avoid environmental pollution, health problems and species loss caused by the overuse of conventional synthetic pesticides, exploration of multi-function agricultural biodiversity to enhance pest management is an important research theme in sustainable agricultural system (Gurr et al., 2003). Intercropping, the agronomic practice for the development of sustainable food production systems (Agegnehu et al., 2006; Eskandari and Ghanbari, 2010), plays an important role in controlling pests and protecting beneficial insects relevant to enhance biodiversity in an agroecosystem (Hassanali et al., 2008; Konar et al., 2010; Smith and McSorley, 2000; Suresh et al., 2010; Vaiyapuri et al., 2010).

The understanding of the mechanisms by which diversification of habitat may favor pest management is important (Garcia and Altieri, 1992; Gurr et al., 2003). Some hypotheses have been put forward to explain why increasing biodiversity in agriculture can lead to suppression of specialist insects. The resource concentration hypothesis and the enemies hypothesis (Root, 1973) are the ones quoted most frequently. The first one states that many phytophagous insects, especially those with a narrow host range, are more likely to find hosts that are concentrated. The "enemies" hypothesis might be further extended to predict that herbivore species diversity would be higher in complex habitats. By rapidly checking outbreaks in these environments, predators and parasites would prevent the potentially dominant herbivore species from monopolizing the available resources. Push-pull strategy involves the behavioural 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 while luring them toward an attractive source from where the pests are subsequently removed (Pyke et al., 1987), and the strategy in exploiting biodiversity has been studied and developed to manage cereal stem borers in maize-based farming systems in eastern and southern Africa (Khan et al., 1997; Khan and Pickett, 2004). The concept was formalized and refined by Miller and Cowles (1990), who termed the strategy stimulo-deterrent diversion strategy (SDDS) while developing alternatives to insecticides for control of the onion maggot *Delia antiqua*. During host plant finding, the searching insects land indiscriminately on green objects such as the leaves of host plants (appropriate landings) and non-host plants (inappropriate landings), but avoid landing on brown surfaces, such as soil. The 'appropriate/inappropriate landings' theory was described by (Finch and Collier, 2000). Plant diversification can be beneficial to control pests via 'top-down' enhancement of natural enemy populations and by resource concentration and other ‘bottom-up’ effects acting directly on pests (Gurr et al., 2003).

The positive contributions of cereals and legumes intercropping/mixing systems in using N sources efficiently (Ghaley et al., 2005), improving land equivalent ratio and system productivity index (Agegnehu et al., 2006), particularly in controlling *Sitobion avenae* Fabricius (Homoptera: Aphididae) by promoting natural enemies (Hassan, 2009; Zhou et al., 2009a; Zhou et al., 2009b) have also been studied in agro-ecosystems. The mechanisms previously described have indicated that diverse backgrounds can affect host selection of herbivores and their natural enemies associated with physical and chemical stimuli from host or nonhost plants. Due to its efficient searching capacity and high predation activity, the multicolored Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) as well as *Episyrphus balteatus* DeGeer, were considered as efficient aphid biological control agents and as the most common and important beneficial insects in fields (Alhmedi et al., 2010b; Francke et al., 2008; Tenhumberg and Poehling, 1995; Verheggen et al., 2008; Verheggen et al., 2007). Semiochemicals are involved in multitrophic interactions, affecting the behaviours of both the herbivores and the beneficial insects. Several volatile molecules are emitted by infested plants but also from the herbivores. In most of previous studies, volatile organic compounds from herbivore-plant associations were assessed on the entomophagous beneficials (Alhmedi et al., 2010a; Alhmedi et al., 2010b; Almohamad et al., 2007; Bahlai et al., 2008; Dicke et al., 2003; Francis et al., 2001; Harmel et al., 2007; Jönsson and Anderson, 2007; Leroy et al., 2010; Obata, 1986; Turlings et al., 1998; Verheggen et al., 2007) whereas less information is available for phytophagous insect responses to healthy versus infested plants.

The objective of this work was to investigate the impact of associating pea to wheat in several combinations (1) on behavioural preference of one aphid pest, namely *S. avenae* and (2) on *H. axyridis* and *E. balteatus* aphidopagous beneficials.

2. Methods and Materials

Plants and Insects

The rearing of *S. avenae* and *A. pisum* were maintained on wheat seedling (cv. Tybalt) and pea seedling (cv. Pois proteagineux) respectively at 22□, 16:8 L:D photoperiod.

Adults of *H. axyridis* were placed in aerated plastic boxes and provisioned with sugar, water-impregnated cotton, and multi-flower pollen. Boxes were placed in controlled environment incubators (16-hr-light photoperiod; 25±2°C).

Adults of *E. balteatus* were reared in cages (75×60 cm and 90 cm high) and were provided with bee-collected pollen, sugar and water.

Cages were maintained in controlled-environment growth rooms (16h light photoperiod; 20±1°C).

Wheat (cv. Tybalt) and pea (cv. Pois proteagineux) for experiments were sown in plastic pots (9×8cm) with 50 tillers and 9 plants, respectively, and were used for experiments at the seeding stage. Plants were grown in a cultured room under similar conditions with aphids.

Olfactometer Assays for *Sitobion avenae*

A two-arm design olfactometer was used to test *S. avenae* preferences for olfactory cues derived from different associations of wheat and pea. It was constructed entirely of Teflon and was closed with a removable glass roof, both previously cleaned with norvanol. The walking arena was 40 cm wide (from center to odor source) and 1.5 cm high (from Teflon walking arena to glass ceiling). Charcoal-filtered air was pushed in each of the olfactometer arms through Teflon tubing and adjusted to 150 ml/min with a digital flowmeter. A pump ventilated the walking arena by removing air from the center at 300 ml/min. A 0.5-l glass chamber was connected to one of the olfactometer arms and was used to dispose of the odor source. Choice was recorded when the subject insect moved past a “choice line” located 5 cm past the center of the walking arena, toward one of the olfactory sources. Aphids not moving past the choice line within 10 min were recorded as nonresponders and excluded from analysis. After every five insects, a clean olfactometer was used. 40 aphids were individually tested for each treatment. The behavioural observations were conducted in a laboratory at 22±1°C and under uniform lighting to avoid interference with insect behaviours.

The following treatment pairs were examined:

(1) wheat plants versus blank control air, (2) pea plants versus blank control air, (3) wheat infested *S. avenae* (prepared by infestation with 50 aphids for a period of 24 hr prior to use) versus blank control air, (4) pea infested *A. pisum* (prepared by infestation with 50 aphids for a period of 24 hr prior to use) versus blank control air, (5) mixed healthy wheat and pea versus blank control air, (6) wheat infested *S. avenae* mixed with healthy pea versus blank control air, (7) mixed healthy wheat and pea infested *A. pisum* versus blank control air, (8) mixed wheat infested *S. avenae* and pea infested *A. pisum* versus blank control air.

Olfactometer Assays for *Harmonia axyridis*

The four-arm olfactometer was used to test *H. axyridis* preferences for olfactory cues derived from wheat and pea as described in 1.2. Eight stimuli were tested on both *H. axyridis* males and females similarly to the treatment pairs used for *S. avenae*. The duration (s) was recorded when the subject insect moved past a “choice line” located 5 cm past the center of the walking arena, toward one of the olfactory sources in 180 s. The behavioural observations were conducted in a laboratory at 22±1°C and under uniform lighting to avoid interference with behaviour of the test insects.

2.4. Visual observations for *Episyrphus balteatus*

Visual observations were conducted in a controlled environment room (22±1°C) fitted with an extraction fan. A net-cage (L×W×H=180×60×90 cm) (Fig. 1) was set up in a black cage (L×W×H= 200×70×100 cm) consisting of a steel frame covered with black cardboard paper to avoid external visual cues. Uniform illumination was provided by four fluorescent light tubes (70 W; Luminux) positioned approximately 10 cm above the net-cage.

Six pots of plant were placed in the net-cage as presented in table 1 and Fig. 1. *E. balteatus* were collected from rearing cages in a separate insectary room. The foraging behaviour of *E. balteatus* was then recorded for 10 min using the Observer® software (Noldus information Technology, version 5.0, Wageningen, The Netherlands). Descriptions of the four behavioural events that were observed were grouped as follows: (1) immobility: the hoverfly was immobilized on the cage without moving; (2) flying cage: the hoverfly hovered in the cage far away the plant; (3) searching: the hoverfly hovered in the cage close to the plant; (4) acceptance: the hoverfly landed on the plant, stayed immobile or walked on it, with proboscis extension on the plant surface; (5) oviposition: the hoverfly female showed abdomen bending and laid eggs. 10 individuals were tested for each treatment. The net cage was cleaned with norvanol and water after each test. Twelve series of dual-choice test bioassay experiments were compared as described in table 1.

2.5. Statistical Analysis

Observed frequencies related to the choice of *S. avenae* in olfactometer assays were compared to corresponding theoretical frequencies by using a $\chi 2$ goodness-of-fit test. A Student's t test was performed to compare the mean duration of *H. axyridis* and mean frequencies of *E. balteatus* responses to the wheat-pea stimuli. All statistical tests were conducted using SAS 9.1 statistical software (Institute, 2001).

3. Results

Sitobion avenae behavioural preferences

The behaviour preference of alatae and apterae of *S. avenae* was observed by the presence of pea odor in wheat plants (Fig. 2). A strong preference was observed for the odor of healthy wheat (χ^2=32.00, P<0.001) and healthy pea (χ^2=24.50, P<0.001). But *S. avenae* alatae was not attracted by odors of wheat infested with aphids and the combination of wheat infested with aphids and pea infested with aphids. However, there were clearly higher proportion of non-responding alatae to odors origining from pea infested with aphids (χ^2=18.00, P<0.001) and the other three combinations: wheat and pea (χ^2=24.50, P<0.001), wheat infested with aphids and pea (χ^2=4.50, P=0.034), wheat and pea infested with aphids (χ^2=12.50, P<0.001).

Harmonia axyridis **behavioural preferences**

The behaviour responses of females and males were observed by the presence of pea tracks in wheat plants (Fig. 3). Females spent more time on aphid infested pea compared to controls (t=2.97 P=0.015), and males stayed less time on healthy wheat when compared to controls (t=-2.36 P=0.042). Although the time spent on treatments also increased in response to the presence of pea tracks in combination with wheat plants both females and males, no significant difference was detected.

Behavioural responses of ***Episyrphus balteatus***

The positive role of pea plants was observed on the different behavioural groups in wheat treatments. The combination of WA, PW and WA induced high frequencies of searching by the *E. balteatus* female compared to the combination of WW, WW and WW (Student's t-test: t = 2.29, P = 0.047) (Fig. 4). There were significant difference in acceptance frequencies of the *E. balteatus* female as follow groups (Fig. 4): PA, PA, PA and WA, WA, WA (Student's t-test: t = 2.42, P = 0.038), WW, PW, WW and WW, WW, WW (Student's t-test: t = 2.22, P = 0.049), WA, PA, WA and WW, WW, WW (Student's t-test: t = 2.43, P = 0.037). Moreover, the oviposition frequencies related to the pea plant infested by related aphid or not were higher than the ones observed with wheat plants (Fig. 4. Student's t-test: test 5: t = 2.43, P = 0.037, test 10: t = 2.38, P = 0.040). For the behavioural observations of *E. balteatus* male, no significant difference was detected except for test 2 in Fig. 5.

4. Discussion

The results of the present investigation show that volatiles from different combination between wheat and pea may affect the behavioural preferences of *S. avenae* and its natural enemies. Plant diversification of agroecosystems can result in increased environmental opportunities for natural enemies and, consequently, improved biological pest control (Altieri and Letourneau, 1982). Intercropping is one opportunity to diversify the crop plant neighbouring if optimal intercrop is selected for push-pull strategy. Semiochemical-mediated host selection has been shown to occur in several species of insect (De Moraes et al., 2001; Han and Chen, 2002; Sema Gencer et al., 2009; Verheggen et al., 2008). Plant chemical and volatile organic compounds (VOCs) defense induction herbivory by chewing insects is mainly regulated by jasmonic acid (Dicke et al., 2009), while infection by herbivory by sucking insects is regulated mainly by salicylic acid (Pieterse et al., 2009). Plant VOC defensive functions include directly deterring herbivores, indirectly attracting natural enemies of attackers (Piesik et al., 2011). It is becoming increasingly clear that a major function of cuticular hydrocarbons in arthropods is to serve as recognition signals. One or more components of the complex mixture of hydrocarbons found on the cuticle of almost all arthropods is often the primary chemical cue (de Vos and Jander, 2010; Howard and Blomquist, 2005).

In our study, the combination of wheat and pea, infested by related aphid to represent all potential situations in fields, improved the frequencies of acceptance and oviposition by *E. balteatus* female. Feeding by insect herbivores induces plants to release chemical signals that serve as important foraging cues for predators, and thus enhance the plants' defense. Predator foraging consists of a series of behaviors that are affected by information about the surroundings. Chemicals are among the main information-conveying agents available to predatory arthropods (Dicke et al., 1990). The location of food, oviposition sites, and suitable microclimates for insects has been linked to the volatile components emitted by other organisms (Kielty et al., 1996). Studies have shown that three compounds (cis-3-hexen-1-ol, linalool, and cis-a-bergamotene) emitted from *Nicotiana attenuata* plants during attack by leaf-feeding herbivores increased egg predation rates by a generalist predator, linalool and the complete blend decreased lepidopteran oviposition rates (Kessler and Baldwin, 2001). The *E. balteatus* foraging and reproductive behaviors according to the volatile emission from aphid-infested plants are also enhanced (Harmel et al., 2007). (*Z*)-3-hexenol and (*E*)-β-farnesene, emitted by aphid-infested plants, induced higher frequencies of *E. balteatus* female searching and acceptance behavior (Alhmedi et al., 2010a; Almohamad et al., 2008), suggesting that selection of the oviposition site by predatory hoverflies relies on the perception of a volatile blend composed of prey pheromone and typical plant green leaf volatiles (Verheggen et al, 2007).

Pea and wheat emitted volatile mixtures were more confident cues for *E. balteatus* leading to improve the efficiency to locate the host plant. As no aphid resources were presented in our experiment, the combination of wheat and pea also improved the frequencies of acceptance and oviposition by *E. balteatus* female. Our findings, that *E. balteatus* significantly prefers cues from healthy wheat or pea plants provide evidence that hoverfly is capable of responding innately to cues from a healthy plants complex. Studies of behavioural responses of adults of *Coccinella septempunctata* to barley and two common barley crop weeds contribute to this result. In a field study, the frequency of adult *C. septempunctata* was higher in barley plots containing high densities of the common weeds *Cirsium arvense* (L.) Scop. and *Elytrigia repens* (L.) Nevski. than in control plots with only barley. In olfactometer experiments in the laboratory, adult *C. septempunctata* showed a significantly more positive response to mixed odours of barley and each of the two weeds than to barley alone (Ninkovic and Pettersson, 2003; Pettersson et al., 2005). The results strongly suggest that olfactory cues and plant-plant communication from diversified plant stands can be important mechanisms in predator attraction to sites with a complex botanical diversity. Glinwood et al also reported Ladybirds *C. septempunctata* were more attracted to combined odours from certain barley cultivars than either cultivar alone (Glinwood et al., 2009). In further study, *C. septempunctata* responded positively to volatiles from aphid-infested barley plants and from previously aphid-infested plants but not to volatiles from uninfested plants or from undisturbed aphids (Ninkovic et al., 2001). In laboratory experiments on adult ladybird orientation to odour from barley, ladybirds were attracted/arrested by the mixed odour of the same barley genotype mixture (Ninkovic et al., 2011). Of 10 corn volatile compounds tested, the twelve-spotted lady beetle, *Coleomegilla maculata* adults responded most strongly to 2-phenylethanol and (*E*)-β-farnesene according to electroantennogram (EAG) responses from the antennae. Two sex pheromone components of aphids, (4aS,7S,7aR)-nepetalactone and (1R,4aS,7S,7aR)-nepetalactol, also elicited significant EAG responses from the antennae of *C. maculate* (Zhu et al., 1999).

Natural enemies are also selective in their feeding, however, and show preferences for certain plant species (Colley and Luna, 2000).

Important variation was observed among natural enemy species in their sensitivity to the VOCs of combination between wheat and pea. In our laboratory test, the combination of wheat and pea had limited effect on the behaviour of *H. axyridis*. Although the time spent on treatments also increased in response to the presence of pea tracks in combination with wheat plants both females and males, there was no significant differences. Field experiments have also shown that cowpea *Vigna unguiculata* were planted in mixtures with millet *Pennisetum glaucum* can not enhance the parasitization rates of *Maruca vitrata*, *Clavigralla tomentosicollis*, and *Ophiomyia phaseoli* and predator-prey ratios of spiders and *Orius* sp (Bottenberg et al., 1998). In a detailed, quantitative review, Andow found that although natural enemy densities tended to be greater in polycultures than in monocultures, only slightly more than half of the 287 herbivore species were consistently less abundant in polycultures (Andow, 1991). One reason for this inconsistent effect of enhanced vegetational biodiversity is that the effects of different types of plants on natural enemies can vary markedly (Colley and Luna, 2000).

We found that *S. avenae* significantly prefers VOCs from healthy wheat or pea plants, but were repellent to VOC cues from a plant-herbivore complex. It has been reported that odors from uninfested maize seedlings were significantly more attractive to the leafhopper, *Cicadulina storeyi* China (Homoptera: Cicadellidae) than odors from *C.*

storeyi-infested seedlings. When tested individually for behavioral activity, the volatile organic compounds (VOCs) of *C. storeyi*-infested seedlings, including methyl salicylate, (*E*)-caryophyllene, (*E*)-β-farnesene, and (*E,E*)-4,8,12-trimethyl -1,3,7,11-tridecatetraene were repellent for *C. storeyi* (Oluwafemi et al., 2011). The behavioural assays also revealed that several volatile compounds are released from herbivore-induced tobacco plants exclusively at night and are highly repellent to female moths (Heliothis virescens) (De Moraes et al., 2001). The odour from the combinations between healthy wheat and pea also reduce the preference of *S. avenae* significantly. These results were consistent with previous studies on barley. Pettersson and his colleague also reported that volatiles from an undamaged barley plant may affect the aphid acceptance of a neighbouring barley plant. This genotype-regulated effect was statistically significant only when certain cultivars were combined (Petterssona et al., 1999).

Manipulation of predator chemical ecology by the inclusion of behavior-modifying compounds in a crop spray mix with reduced amounts of insecticide may allow for effcient aphid control with less environmental contamination. Our focal-insect observations were consistent with results from wheat-pea intercropping in field and suggested that short-term, behavioural studies may help predict the occurrence of aphids and its natural enemies at larger spatial and temporal scales. Further works have to focus on the effect of the combination between wheat and pea on other entomophagous beneficials (eg. lacewing, aphid parasitoids and so on) that also constituted the important natural enemies of cereal aphids in field. The use of such a wheat-pea system may be a promising tool in aphids control to reduce the chemical dependency in agroecosystems, and could enhance the syrphid occurrences to contribute to the augmentative biocontrol through a natural way of preventing aphids. Our findings allow us to positively consider the pea – wheat association for further field assays due to the contribution of pea plants in wheat system, which makes them unattractive for the insect targeted pest and attractive to natural enemies.

5. Acknowledgements

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Figures and tables

Figures

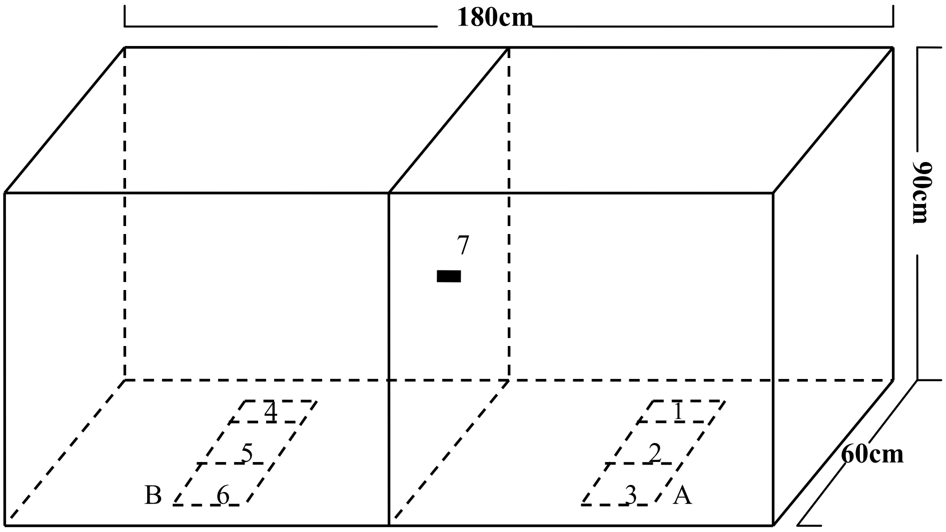

Fig.1. Schematic of net-cage used for assays examining the behaviour of *Episyrphus balteatus* in response to cues originating from wheat and pea. 1, 2, 3, 4, 5 and 6 represented sites where pots of plant placed, 7 represented site where *E. balteatus* was released, A: the combination of plants A, B: the combination of plants B.

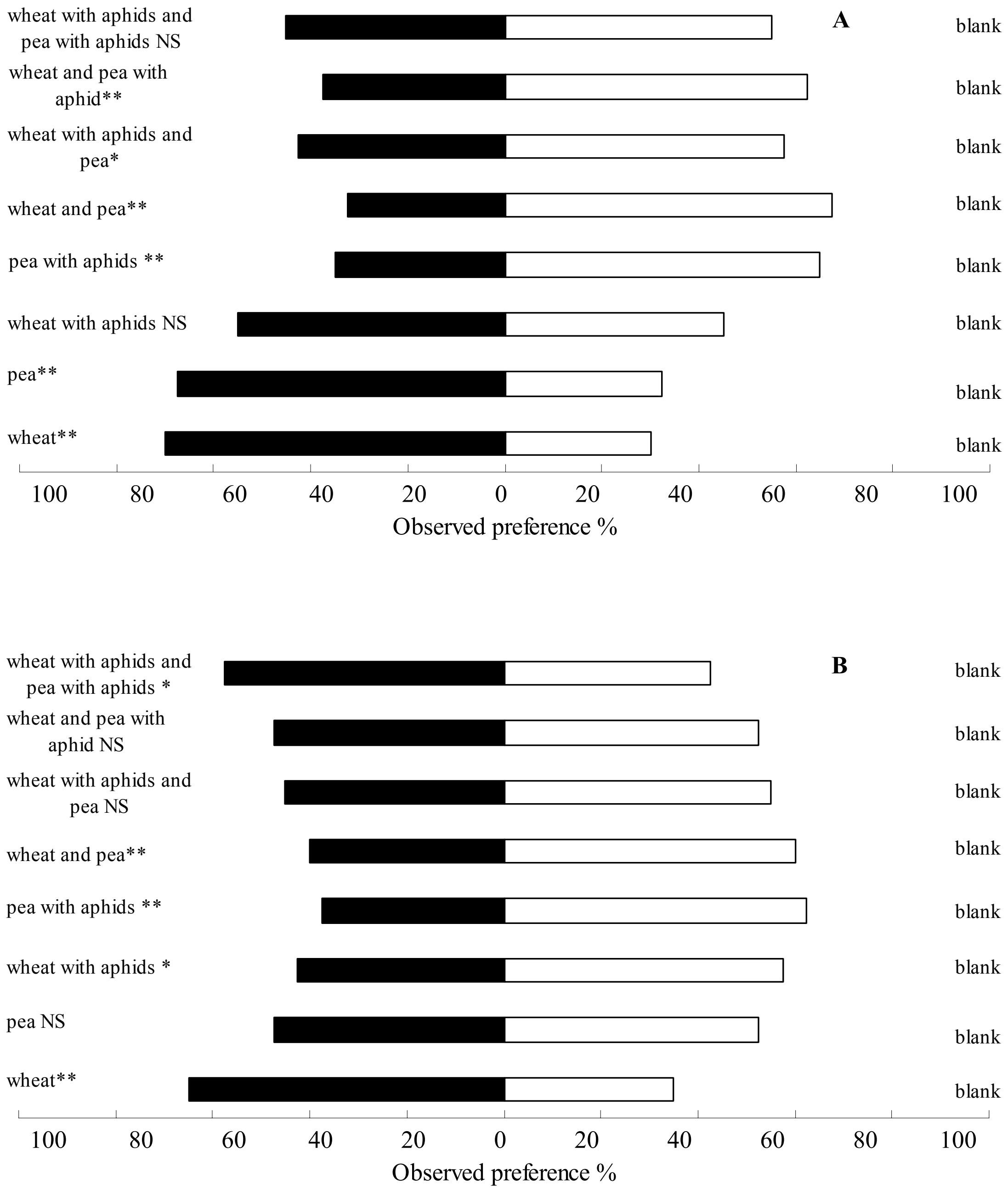

Fig.2. Behavioural responses of the grain aphid, *Sitobion avenae*, to wheat and pea (A: alatae B: apterae) Chi-square analysis, $^{**}P<0.01$, $^{*}P<0.05$, NS: not significant.

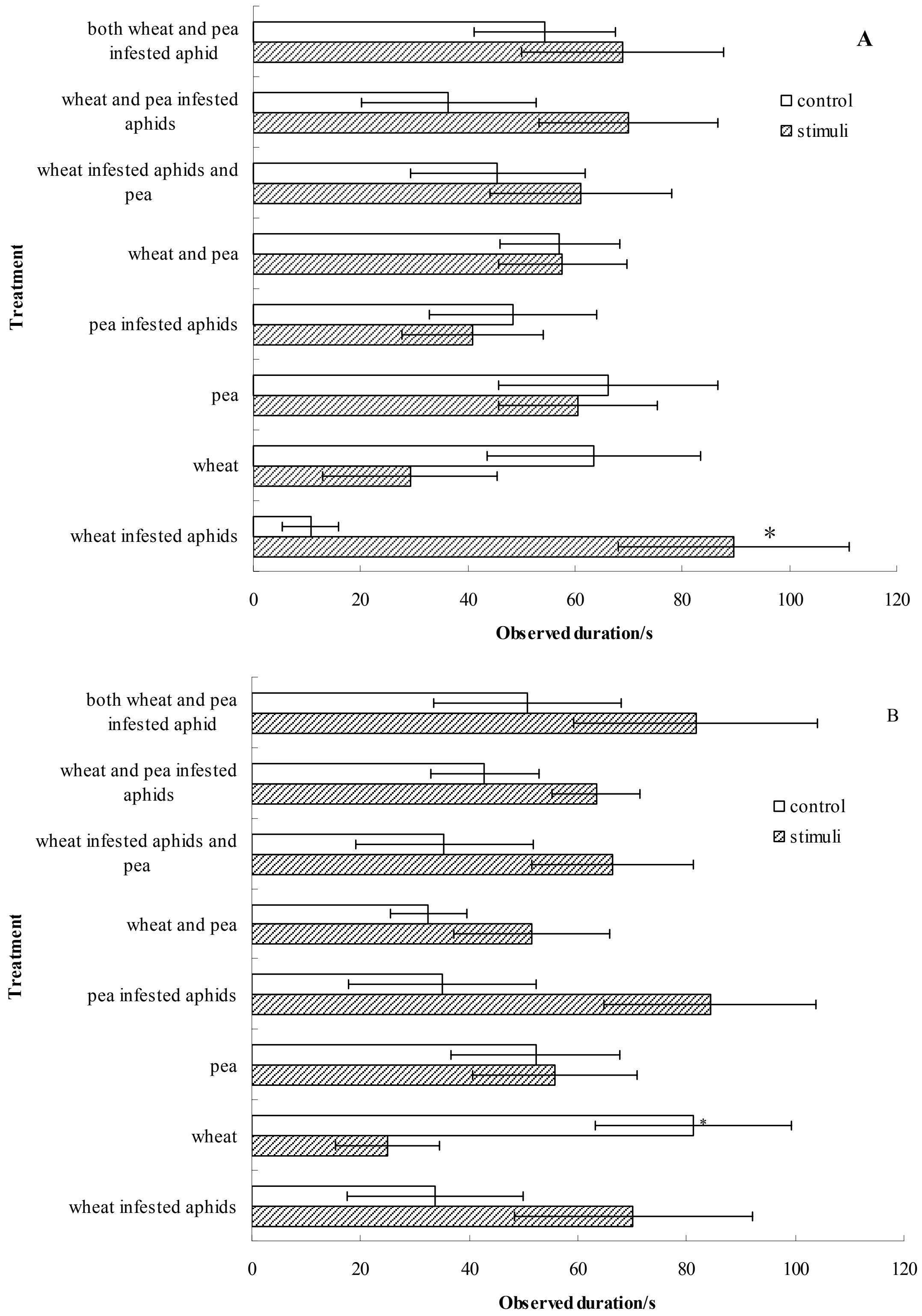

Fig.3. Mean durations ± SE of behavioural observations of *Harmonia axyridis* females in response to wheat-pea tracks in dual-choice experiments (A: female B: male). t paired test, *$P<0.05$.

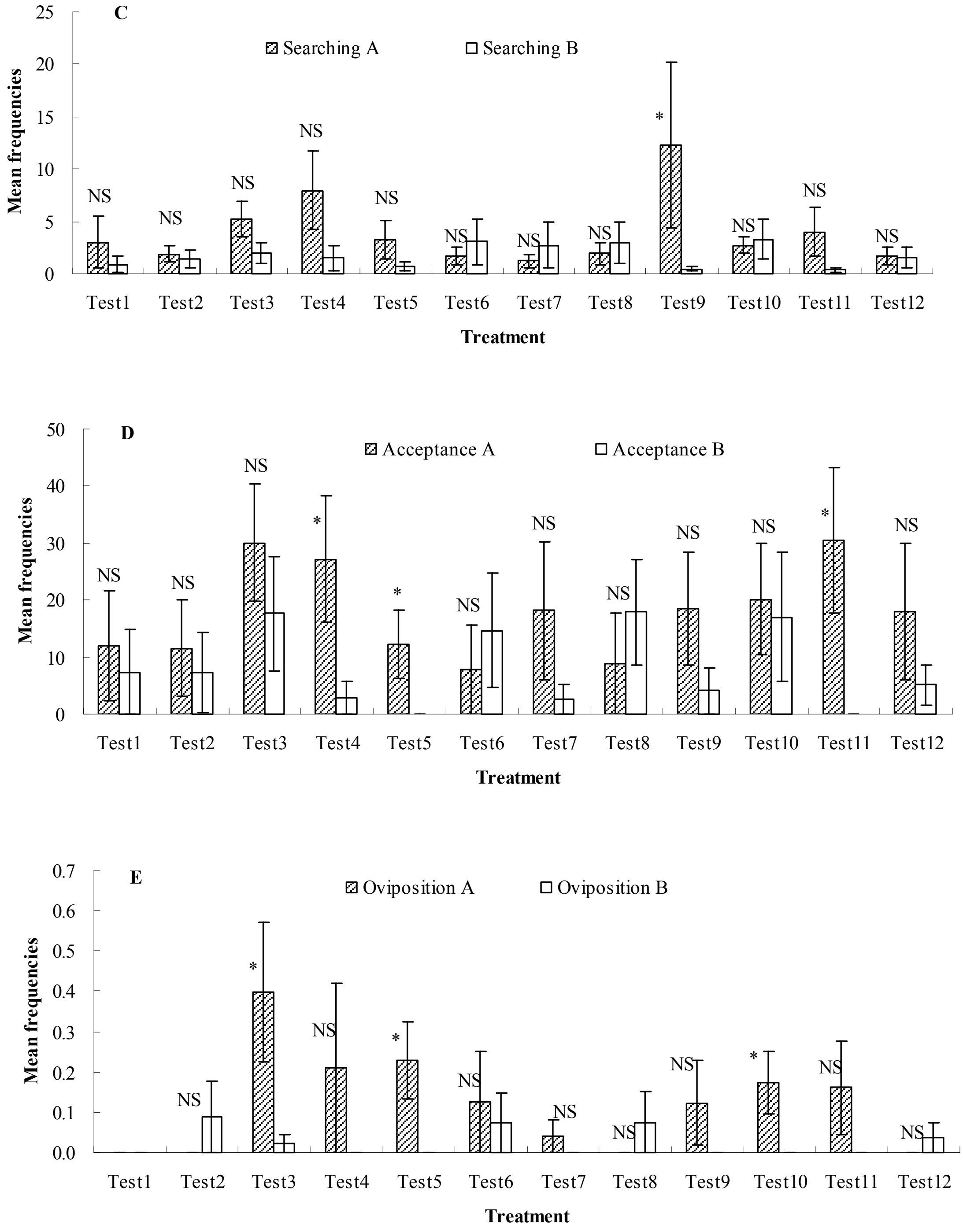

Fig.4. Behavioural observations (mean frequencies ± SE) of *Episyrphus balteatus* females in relation to wheat and pea in dual-choice experiments (C: searching, D: acceptance, E: oviposition); NS and * indicate no significant and significant differences at $P<0.05$ (n = 10).

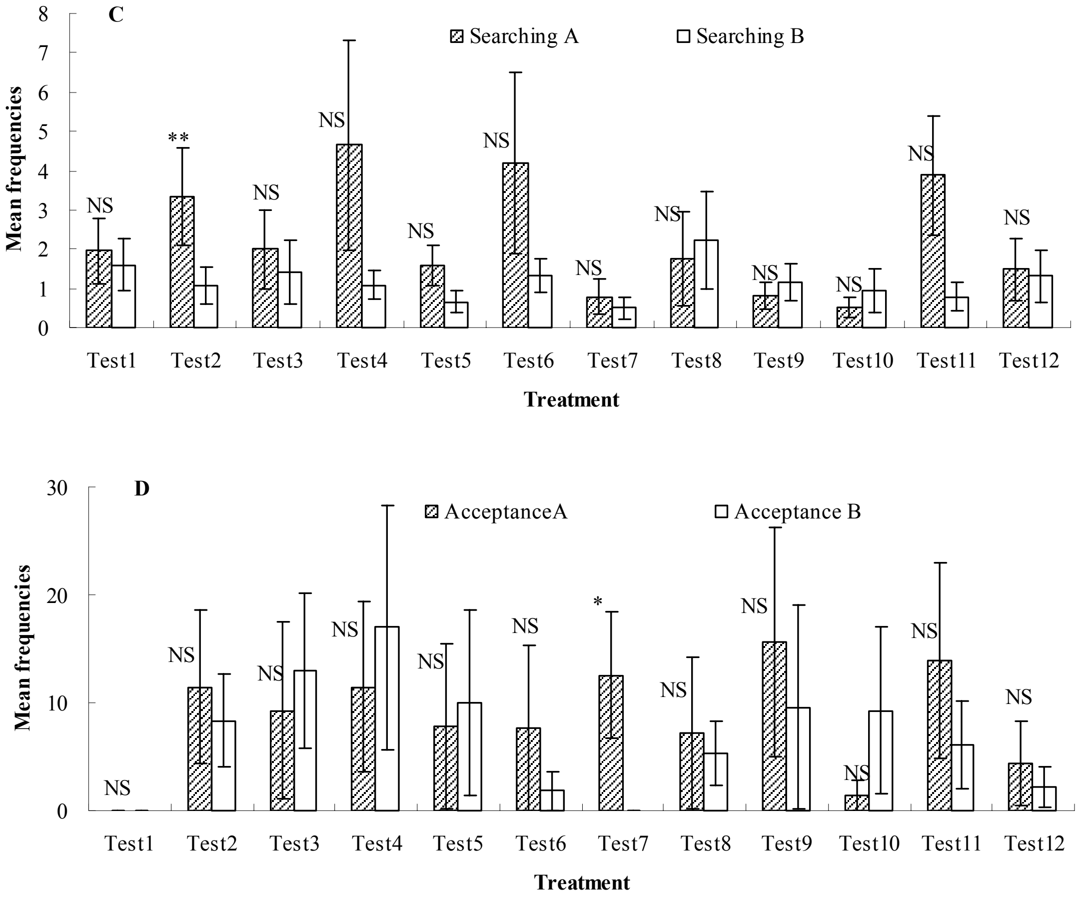

Fig.5. Behavioural observations (mean frequencies ± SE) of *Episyrphus balteatus* males in relation to wheat and pea in dual-choice experiments (C: searching, D: acceptance); NS and ** indicate no significant and significant differences at $P<0.01$ (n = 10).

Tables

Table 1 The different model (combination) between wheat and pea[a]

series		**A**			**B**	
	1	**2**	**3**	**4**	**5**	**6**
Test1	PW	PW	PW	WW	WW	WW
Test2	PW	PW	PW	WA	WA	WA
Test3	PA	PA	PA	WW	WW	WW
Test4	PA	PA	PA	WA	WA	WA
Test5	WW	PW	WW	WW	WW	WW
Test6	WW	PW	WW	WA	WA	WA
Test7	WW	PA	WW	WW	WW	WW
Test8	WW	PA	WW	WA	WA	WA
Test9	WA	PW	WA	WW	WW	WW
Test10	WA	PA	WA	WA	WA	WA
Test11	WA	PA	WA	WW	WW	WW
Test12	WA	PW	WA	WA	WA	WA

[a] PW: one pot of pea without aphids, PA=one pot of pea infested with aphids (50 ints), WW=one pot of wheat without aphids, WA=one pot of wheat infested with aphids (50 ints)

A and B represent zone A and B respectively, 1,2,3,4,5 and 6 represent the number of site in net-cage.

VI.2. Adaptation of wheat-pea intercropping pattern in China to reduce *Sitobion avenae* (Hemiptera: Aphididae) occurrence by promoting natural enemies

Haibo Zhou[a,b], Julian Chen[a], Yong Liu[c], Frédéric Francis[b], Eric Haubruge[b], Claude Bragard[d], Jingrui Sun[a]

[a] State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China

[b] Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux, 5030, Belgium

[c] College of Plant Protection, Shandong Agricultural University, Taian, 271018, PR China

[d] Université catholique de Louvain, Earth & Life Institute, Applied Microbiology Phytopathology, Louvain-la-Neuve, 1348, Belgium

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Abstract: Crop intercropping as a strategy of increasing biodiversity in fields could reduce pest damage and improve the crop production. Here, a study was undertaken to evaluate the role of different wheat-pea intercropping patterns in conserving arthropod natural enemies and suppressing cereal aphids in agroecosystems. Wheat - pea intercropping increased the abundance of natural enemies when compared to wheat monoculture with a higher effect planting an 8-2 pattern of wheat and pea respectively. Wheat-pea intercropping preserved and augmented natural enemies more than a monoculture of wheat. Highest abundance of ladybeetles in 2008 and 2009 were occurred in the 8-2 pattern, followed by in the 2-2 and 4-2 wheat-pea intercropping patterns, and wheat monoculture pattern. The highest densities of aphid parasitoids were found in the 4-2 pattern in 2008 and the 8-2 pattern in 2009. Spatial distribution of the aphid population in the peak stage was spatially heterogeneous, and highest density of aphids was shown visually in wheat monoculture field. The Land equivalent ratio, 1.121-1.187 for wheat-pea intercropping in 2008 and 1.114-1.174 for wheat-pea intercropping in 2009, showed that intercropping of wheat and pea has potential to improve the utilization of plant growth resources as compared to sole crops. Wheat-pea intercropping could contribute to control English grain aphids effectively by enhancing the density of natural enemies, especially with an 8-2 row pattern of wheat-pea respectively.

Key words Wheat, Pea, *Sitobion avenae*, Agro-biodiversity, Natural enemies

1. Introduction

In the developing world, agricultural diversity has been eroded as monocultures dominate (Altieri & Nicholls, 2004). Genetically homogeneous crop monocultures could enhance the evolution, multiplication and spread of newly adapted weed, pest insect and pathogen on massive and uniform crop areas (Finckh & Wolfe, 2006; Karban & Baldwin, 1997). Crop intercropping as a traditional agricultural technique for preventing crop yield decrease from plant disease and pests infestation in different world geographical areas (Altieri, 1999; Ma et al., 2007; Trenbath, 1993), can also increase biodiversity in fields to encourage environmentally sustainable agricultural production with low inputs of pesticides (Ghaley et al., 2005). Many reports on the relationship between insect species diversity and functioning of natural enemy assemblages lead to promote pest control in agroecosystems by crop-associated biodiversity increase (Finke & Denno, 2004; Hummel et al., 2009; Keeping et al., 2007; Khan et al., 1997; Lin et al., 2003; Ninkovic et al., 2011). Intercropping has also been described potentially increasing crop yields by suppressing pest outbreaks (Mucheru-Muna et al., 2010; Rao et al., 2010; Smukler et al., 2010; Vaiyapuri & Amanullah, 2010).

Wheat is one of the most important cereal crops for the people of the world as well as China, and English grain aphid, *Sitobion avenae* Fabricius (Homoptera: Aphididae), is one of the most dominant and destructive pest insects in wheat production regions in China (Cai et al., 2004; Zhao et al., 2009). *S. avenae* causes severe damage to wheat by feeding on leaves and developing ears, as well as be an efficient barley yellow dwarf virus transmission vector both within and between crops (especially strains BYDV-PAV and BYDV-MAV) (Van Emden & Harrington, 2007). These habitats of biodiversification may provide increased resources such as alternative prey/hosts, pollen and nectar for parasitoids and predators from flowering plants (Baggen & Gurr, 1998; Irvin & Hoddle, 2007; Landis, 1994).

The widespread application of geographical information systems (GIS) in agriculture for the spatial analysis of insect pests (Byrne et al., 1996; Merrill et al., 2009; Peng & Brewer, 1994) was developed to quantify the spatial distribution of insect pests and improve forecasting and risk assessment of outbreaks. A spatially explicit Russian wheat aphid, *Diuraphis noxia* density were analyzed and compared using GIS to delineate *D. noxia* distribution within the winter wheat agroecosystem (Merrill et al., 2009). Such the model created by GIS softwore could be applied to generate risk assessment maps predicting areas of high *D. noxia* densities during the early spring.

One solution could be to diversify agroecosystems by increasing the number of species grown and using more leguminous crops (Altieri, 1999; Malézieux et al., 2009). In China, the maintenance of pea cover between rows of wheat crop reduced populations of insect pests，*Sitobion avenae* (Fabricius) and enhanced the population and richness of natural enemies(Zhou et al., 2009a; Zhou et al., 2009b). Also, legume intercrops are also potential sources of plant nutrients that complement/supplement inorganic fertilizers by direct nitrogen transfer from the legume to cereal (Giller & Wilson, 1991). Altieri (1999) also suggested that correct biodiversification results in pest regulation through restoration of natural control of insect pests, diseases and nematodes and also produces optimal nutrient recycling and soil conservation by activating soil biota, all factors leading to sustainable yields, energy conservation, and less dependence on external inputs. In this study, the population dynamics of the English grain aphid and its natural enemies in different wheat-pea intercropping patterns and wheat monoculture were investigated and compared. A model illustrating the spatial distribution of English grain aphid densities during peak period was analyzed and compared using ArcGIS and the effects of wheat-pea intercropping patterns on crop yields were also assessed.

2. Materials and Methods

Wheat and pea varieties

The wheat cultivar (*Triticum aestivum*) used in the study is cv. Beijing 837 which is currently planted commercially in Huang-Huai-Hai plain, China. Pea variety (*Pisum sativum*) cv. Zhongwan-5, provided by Institute of Beijing Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS), was used in the study.

Field experimental plots

Field studies were carried out at the Langfang Experimental Station of Plant Protection Institute, CAAS, Hebei Province of China (39°30′N, 116°37′E) in 2008 and 2009. Four planting patterns were compared: three wheat-pea intercropping patterns changing the relative density of pea from 2-2, 4-2 and 8-2 pattern (each number representing the number of rows of wheat followed by the row number of pea respectively) and a fourth constituting of a wheat monoculture control without intercropping. Each treatment plot was 66.7 m^2 (10×6.67m). Treatments were replicated three times in a completely randomized design within the field. A 4 meter wide area was set around the plots to decrease potential border effects on insect dispersion.

Wheat and pea were sowed at the rates of 6,000,000 wheat seeds and

400,000 pea seeds per ha according to the differential wheat-pea row density intercropping pattern with 20 and 40 cm distance between rows in wheat and pea respectively. Wheat was sowed in October of each year followed by pea in March of the next year. Wheat and pea were harvested in June. No pesticides and herbicides were applied on the fields during the whole growth stage of wheat and pea. All plots were irrigated during the growing season as standard agronomic practices used in northern China.

Sampling of arthropod species

S. avenae apterae was observed in five "one square meter plot" which were chosen homogeneously in each plot. Within each sampling plot, thirty randomly selected wheat tillers were used as one sampling unit. Moreover, *S. avenae* alatae were sampled using yellow sticky traps (30×20 cm) (Wang et al., 2009; Zhu & Park, 2005), which was individually fixed on a bamboo stake 1.2 m above the ground in the center of each plot. The number of *S. avenae* alatae was assessed from the yellow sticky traps which replaced by a new trap after each counting. Lady beetles (all stages) on all wheat plants within the "one square meters plots" squares covering three rows of wheat were counted and identified in the center of each plot. Aphid parasitoids were collected using an insect net trap (30cm diameter×50cm long) in three rows at the center using 20 sweeps per plot and identified under a dissecting microscope in the laboratory. Insects were sampled in field every 5 days from 12th April to 6th June in 2008, and from 12th April to 27th May in 2009.

Spatial distribution of aphids

Arc GIS 9.2 was used to map aphid spatial distribution (Liebhold et al., 1993). Ordinary Kriging (Fievet et al., 2007; Krige, 1966; Matheron, 1963) was applied to compare aphid densities at peak abundance among plot treatments.

Crop yields

Yields of wheat and pea were assessed by harvesting and weighing crop products from each plot leading to the calculation of yields in kg $.ha^{-1}$ after drying in the sun. The Land Equivalent Ratio (LER), defined as the relative land area required as sole crops to produce the same yields as intercropping, is a measurement of the resource utilization efficiency for an ecological system (Mead & Willey, 1980) and was calculated for wheat-pea intercropping as the sum of the partial LER values for wheat (L_w) and pea (L_p). The LER values were calculated as follows: $LER=L_w+L_p=Y_{iw}/Y_{mw}+Y_{ip}/Y_{mp}$. Where Y_{iw} and Y_{ip} are yields of crops 'wheat' and 'pea' in intercropping, Y_{mw} and Y_{mp} are yields of crops 'wheat' and 'pea' in monoculture. When LER values are higher than 1, it indicates an advantage from intercropping in terms of the use of environmental resources for plant growth.

Statistical analysis

The density of insects was compared among plot treatments using a one-way analysis of variance (ANOVA) (Institute, 2001) followed by Tukey's significant differences test (HSD). Effects of years and planting patterns on aphid and related natural enemies were analyzed using General Linear Model (GLM) procedure. Where necessary, the raw data used in ANOVA and GLM were transformed using $\sqrt{x+1}$ or $\sqrt{x}$ to meet assumptions of normality.

3. Results

Aphid and main natural enemy abundance in different wheat–pea intercropping patterns

Significant differences in apterae and alatae populations of the *S. avenae* among the four planting patterns in 2008 and 2009 were observed (Fig. 1 A, B) (for apterae in 2008: F=10.81, df=3,8; P<0.01; in 2009: F=38.09, df=3,8; P<0.01; for alatae in 2008: F=135.74, df=3,8; P<0.01; in 2009: F=43.66, df=3,8; P<0.01). The population of apterae aphid was significantly higher in wheat monoculture than in 4-2 and 8-2 patterns in 2008, and also higher than in other patterns in 2009. Populations of alatae aphids were significantly higher in wheat monoculture than in all intercropping patterns in 2008 and in 2009 excepting in the 4-2 intercropping pattern the latter year.

Generally, natural enemies of aphid were significantly more abundant in all intercropping patterns than in wheat monoculture in 2008 and 2009. The highest abundance of ladybeetles occurred in the 8-2 pattern in 2008, followed by in the 2-2, 4-2 patterns and wheat monoculture plots. Similarly, a significant difference for ladybeetles in 2009 can be detected among planting patterns (Fig. 1 C) (in 2008: F=24.63, df=3,8; P<0.001; in 2009: F=22.75, df=3,8; P<0.001). The aphid parasitoids densities in wheat monoculture pattern were also significantly lower than that in other patterns (Fig. 1 D) (2008: F= 62.38, df=3,8; P< 0.001; 2009: F=81.13, df=3,8; P <0.001), and the highest densities of aphid parasitoids were observed in the 4-2 and 8-2 intercropping patterns in 2009 and 2008 respectively.

Spatial distribution of aphid during peak period

Within field scale, *S. avenae* distribution was spatially heterogeneous (Fig. 2 A, B). The range of *S. avenae* densities in the whole field could be visualized by overlapping the maps. Highest aphid density congregated around the centre of wheat monoculture pattern field in 2008 and 2009. Lowest densities of aphids in 2008 were found in 8-2 intercropping pattern and in 2-2 pattern, the same phenomenon was also observed in 2009.

Effect of years and planting patterns on the abundance of *S. avenae* and its natural enemies

The interaction of abundances of *S. avenae* apterae and alatae*,* lady beetle and aphid parasitoid between the 2 years and among the planting patterns was analyzed (Table 1). No significant interaction between years and planting patterns was observed on the ladybeetle abundance, but a significant interaction between these factors was determined on the *S. avenae* apterae and alatae, as well as aphid parasitoids.

Crop yields

Wheat - pea intercropping significantly increased the wheat and pea yield in both years when compared to monoculture yields (Table 2). In 2008, the highest wheat yield was obtained in the 2-2 intercropping pattern (6940±213 kg ha^{-1}), followed by in the 8-2 and 4-2 intercropping patterns, while wheat monoculture had the lowest yield (5448±100 kg ha^{-1}). Pea yields increased from 1593±136 kg ha^{-1} in monoculture to 1881±456 kg ha^{-1} in the 8-2 intercropping pattern. Land use efficiency of different wheat-pea intercropping patterns increased from 12.1% to 18.7%. In 2009, the highest wheat yield was recorded in the 2-2 intercropping pattern, followed by the ones from the 8-2, 4-2 and wheat monoculture patterns. Pea yields were significantly higher in intercropping than in monoculture pattern. LER value related to wheat-pea intercropping patterns increased from 11.4% to 17.4%.

4. Discussion

The exacerbation of most insect-pest problems has been associated with increases in crop monocultures at the expense of the natural vegetation, thereby decreasing local habitat diversity. Within-field plant diversity can be manipulated by designing polycultures of various temporal and spatial crop arrangements (Altieri & Letourneau, 1982). Intercropping is one of the strategies to increase plant heterogeneity and beneficial diversity in agricultural systems leading to control insect pests and crop damages (Andow, 1991b; Hassan, 2009; Tahvanainen & Root, 1972). Indeed, cowpea-sorghum intercropping was already demonstrated to significantly reduce aphid populations when compared to sole cowpea crops (Hassan, 2009). Wheat-oilseed rape intercropping was also provided an example of a more efficient aphid population control than in wheat fields only (Wang et al., 2009). Here, our results confirmed that another intercropping system is also efficient when combining pea and wheat crops to reduce aphid pest population to obtain higher yields. Several studies have shown that it is possible to stabilize the insect communities of agroecosystems by designing and constructing vegetational architectures that support populations of natural enemies or have direct deterrent effects on pest herbivores. Some modified factors can explain the decrease of aphid population growth rates in intercropping patterns: (1) wheat-pea intercropping preserved and increase the predatory and parasitoid beneficial populations such as the ladybeetle and braconid wasps respectively; (2) pea and wheat emitted volatile mixtures were less confident cue for both wheat and pea infesting aphids leading to a changes in aphid behavior and efficiency to locate the host plant; (3) pea can act as a wide barrier and interfered in the cereal aphid movements from overwintering host plants around the crop plots to wheat crops.

The distribution maps with aphid density isoclines revealed spatial changes on wheat field, and aphid intense aggregation distribution. The spatial distribution of *S. avenae* in peak time was relatively heterogeneous and similar aggregation distribution in all planting plots was found. The spatial separation of sampling points prevented any detailed analysis of distribution relative to aphid colonies on individual plants. Obviously, three red areas in figure 2 in both years strongly and visually suggested that homogeneous crops often lead to adaptations of herbivores to plant defenses with large aphid density. At the same time, the risk assessment of aphid outbreak could be done based on the density maps which could indicate the area with high, medium, and low risk for aphid population and the trend of expansions.

Increasing plant-biodiversity in agroecosystems could easily lead to higher pest control by natural enemies (Andow, 1991a). The densities of aphid natural enemies on wheat were significantly different among the different treatments, correlating with differences in natural enemies populations in the intercropping treatments. The higher beneficial density associated with the reduction of aphid populations was found in intercropping patterns. These results were in accordance to the natural enemies hypothesis which suggest that natural enemies are more abundant in diversified habitats where they can impose higher mortality on herbivores than in monocultures (Bach & Tabashnik, 1990; Root, 1973). The combined effects of intercropping and natural enemies lead to the best control of English grain aphid on 4-2 and 8-2 intercropping patterns. The addition of floral resources can enhance parasitoid longevity, fecundity, searching activity, parasitism rates, and increase female sex ratios so as to increase their effectiveness (Berndt & Wratten, 2005; Landis et al., 2000; Tylianakis et al., 2004). It is possible to control English grain aphid by using floral plants to design an ideal intercropping system and consequently to reduce the chemical dependency in agroecosystems.

The densities of natural enemies were found to rise and fall being in conformity with the trends of aphid development significantly in 2008, but this phenomenon which was not proved in 2009, should be further studied. In diversified habitats, the presence of floral resources could benefit natural enemies in a number of ways by providing shelter, as a source of alternative hosts or prey, or by providing non-host foods such as nectar and pollen (Frere et al., 2007; Langellotto & Denno, 2004). Our data showed that intercropping patterns that can increased crop diversity in the agroecosystems affected the abundance of natural enemies. These results support the resource concentration hypothesis that specialist herbivores are more likely to find, stay, and reproduce in pure than in diverse stands (Root, 1973). In addition, the interaction between years and planting patterns on aphids can also be affected by the meteorological conditions yearly and the proportion of pea plant in overall intercropping system, and the corresponding change in interaction on the natural enemies could be accompanied by influence of aphids' occurrence.

Biodiversity in agricultural lands could be conducive to minor loss of production area (Smukler et al., 2010). The experimental results underlined here an interesting wheat-pea intercropping application to increase both wheat and pea yields when in association, resulting in an increasing field utilization efficiency. The analysis of yield data suggests that intercropping can effectively improve the land utilization ratio, and using intercropping strategy in insect management programs will be profitable and help farmers to increase wheat (or other intercropping crops) yields and to reduce insecticide usage.

Our study suggests that wheat-pea intercropping following by temperate patterns could be used in crop protection strategies. Summary the suppression of aphid population, increasing natural enemies and enhancing crops yield by different patterns of wheat-pea intercropping, the 8-2 pattern (wheat-pea intercropping by 8 and 2 rows) was found to be the optimization model in the field. The prospects for understanding and exploiting the intercropping have advanced rapidly, particularly with the discovery the relationship among biodiversity, stability of agroecosystem, herbivorous insect and natural enemies.

5. Acknowledgments

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Figures and tables

Figures

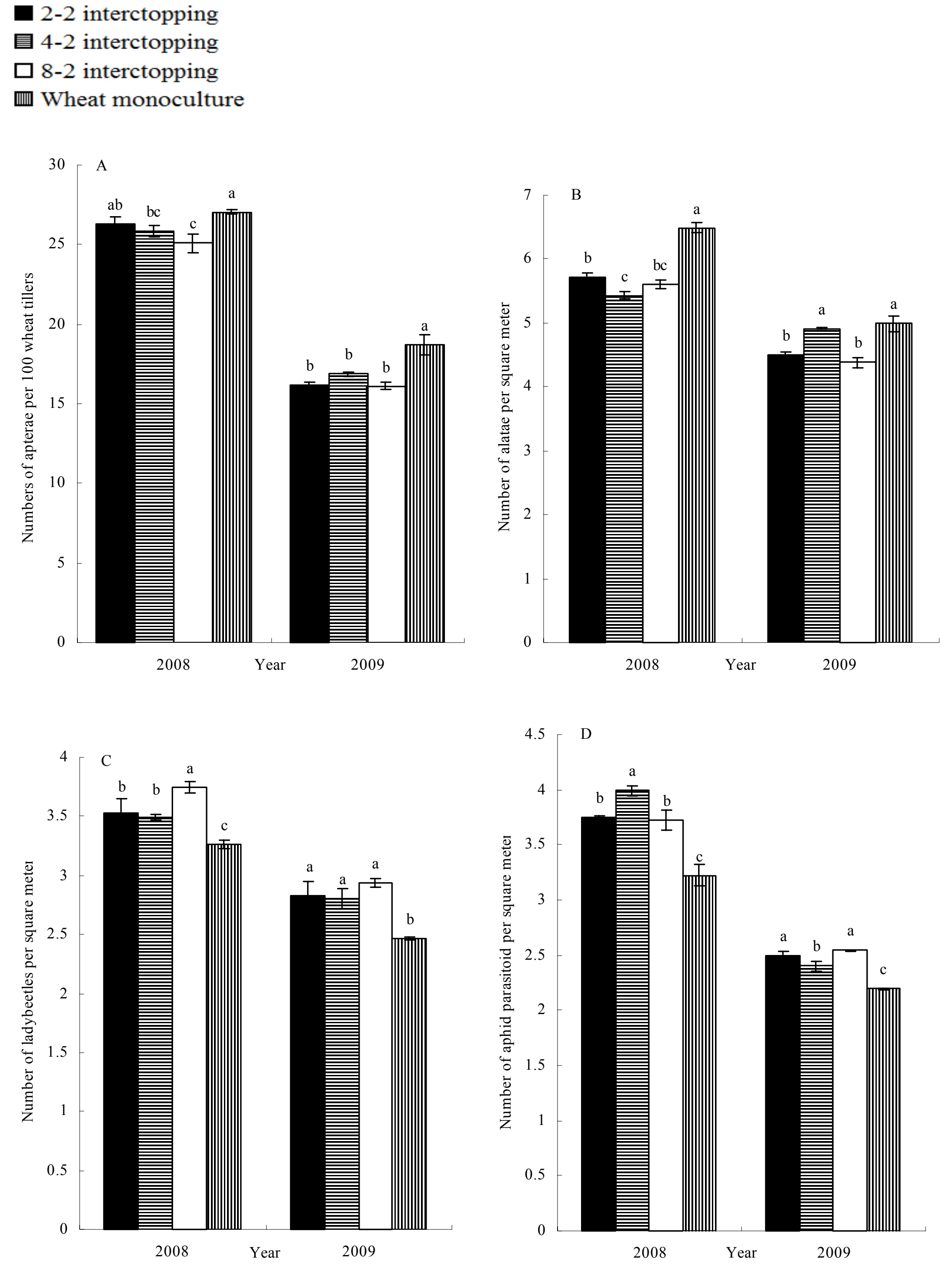
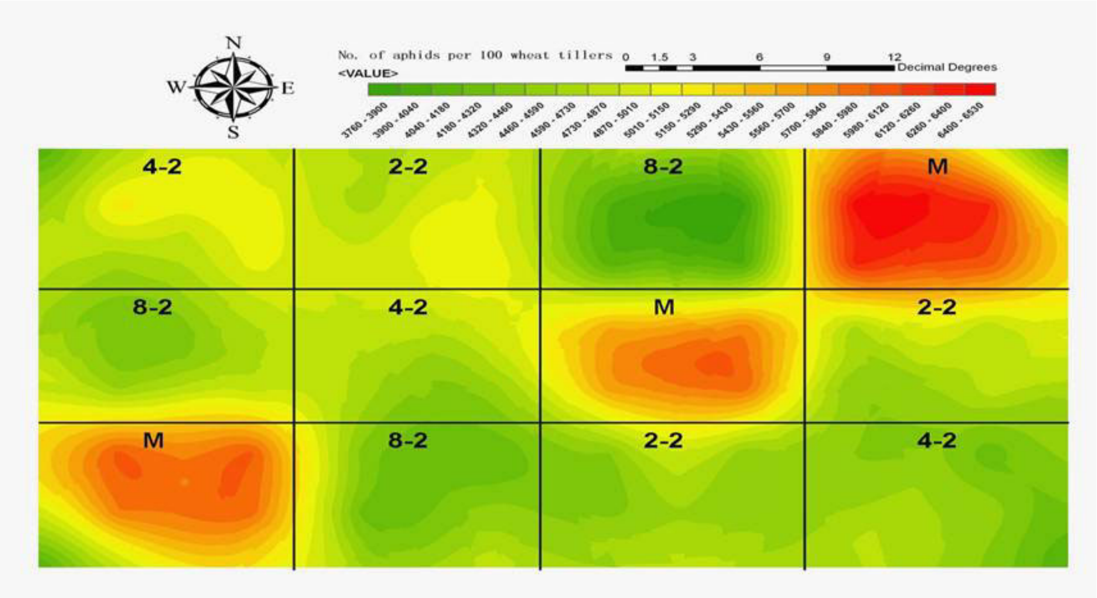


Figure 1 Abundance (mean± SE) of *Sitobion avenae* and related natural enemies.

(A) apterae aphids. (B) alatae aphids. (C) predatory ladybeetles (*Coccinella septempunctata*

(L), *Harmonia axyridis* (Pallas) and *Propylaea japonica* (Thunberg)). (D) aphid parasitoids (*Aphidius avenae* (Haliday) and *Aphidius gifuensis* (Ashmead)). Within a year, bars topped by different letters are significantly different at P<0.05

(A)



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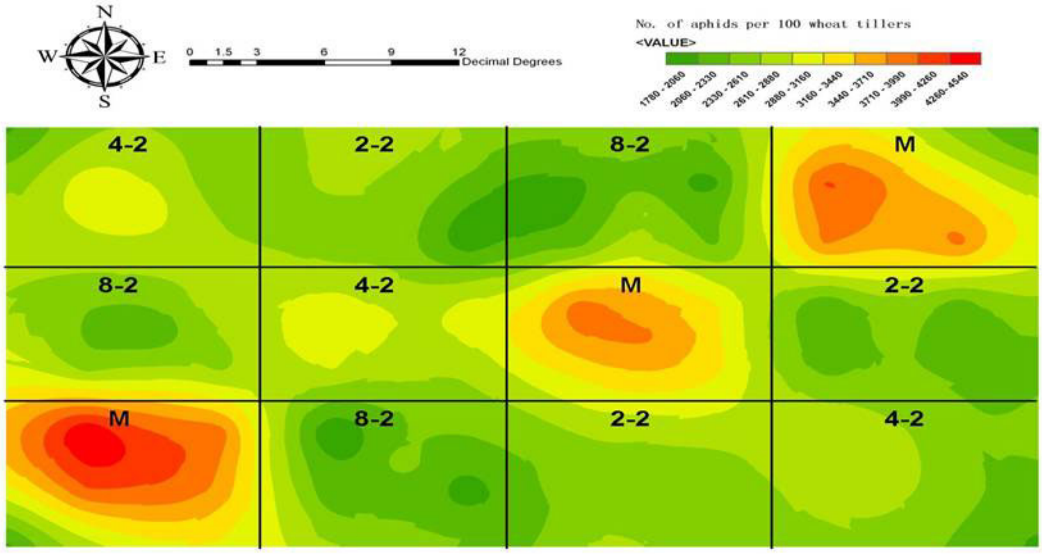

Figure 2 Maps of *Sitobion avenae* densities estimated during peak period (2008:12-27 May; 2009:2-17 May). (A) GIS image of aphid population in 2008. (B) GIS image of aphid

population in 2009.

Tables

Table 1 F-test on effect of year and planting patterns on the abundance of *S. avenae* and natural enemies on wheat

Source of variation	d.f.	*S. avenae* apterae		*S. avenae* alatae		Ladybeetles		Aphid parasitoids	
		F	P	F	P	F	P	F	P
Year	1	3319.54**	<0.0001	1205.03**	<0.0001	673.19**	<0.0001	5427.69**	<0.0001
Intercropping pattern	3	37.54**	<0.0001	108.29**	<0.0001	46.46**	<0.0001	170.02**	<0.0001
Year*Intercropping pattern	3	5.56*	0.01	42.02**	<0.0001	1.14NS	0.367	47.56**	<0.0001

******P***<0.05, *****P***<0.01, NS, not significant**

Table 2 Crop yields (mean ±SE) in different wheat-pea intercropping patterns in 2008 and 2009

Patterns	2008			2009		
	Wheat (kg/ha)	Pea (kg/ha)	LER	Wheat (kg/ha)	Pea (kg/ha)	LER
22 Pattern	6,940±213(3239)aA	1,770±171(944)abA	1.187	5,640±204(2632)aA	1,520±87(811)bA	1.114
42 Pattern	6,032±190 (3734) bB	1,822±86(694)abA	1.121	5,386±173(3334)aA	1,787±176(681)aA	1.174
82 Pattern	6,197±31(4695) bB	1,881±87(456)aA	1.148	5,493±180(4162)aA	1,576±104(382)abA	1.144
monoculture pattern	5,448±100cC	1,593±136bA		4,715±228bB	1,460±102bA	

Means within a column followed by the different small letter show the significant difference at *P*<0.05, Means within a column followed by the different capital letter show the significant difference at *P*<0.01.

VI.3.Mixing crops and intercropping as efficient aphid control strategies: illustration with pea-wheat association

Haibo Zhou(1,2), Julian Chen(2), Yong Liu(3), Bernard Bodson (4), Eric Haubruge(1), Dengfa Cheng(2), Frédéric Francis(1)

(1) Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux, 5030, Belgium

(2) State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China

(3) College of Plant Protection, Shandong Agricultural University, Taian, 271018, PR China

(4) Crop production, Gembloux Agro-Bio Tech, University of Liege, Gembloux, 5030, Belgium

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Abstract

Field experiments were performed to associate two crops, wheat and pea, exploiting plant association as habitat management to enhance biological control of aphids within both crops. The diversity and abundance of aphids and their natural enemies were investigated using water yellow traps and visual observations in wheat and pea grown each alone, mixed in the same plots and wheat intercropped with pea. Populations related to both crop species, *Metopolophum dirhodum* (Walker) and *Sitobion avenae* (Fabricius) in wheat but also *Acyrthosiphon pisum* Harris in pea obviously decreased when crop were grown in association. Aphidophagous predators, *Episyrphus balteatus* De Geer, *Chrysoperla carnea* Stephens and *Harmonia axyridis* Pallas were the predominant beneficial species in cereal fields. In water yellow traps, 46.1% of the collected aphidophagous predators were lacewing, followed by hoverflies, (43.1%), and ladybirds (10.8%). The high abundance of hoverflies, lacewings and ladybirds were found in wheat mixed with pea field, then in wheat intercropped with pea fields, more than in wheat and pea each alone. In addition, beneficial insect abundance in wheat-pea mixing or intercropping fields increased significantly in the latter half of the season. Our findings are discussed in relation to the use of combining plants as an alternative strategy in habitat crop management for efficient and sustainable pest control.

Key words: wheat, pea, habitat management, aphid control

1. Introduction

In many agroecosystems, landscape structural diversity has been greatly simplified and insect communities are dominated by pest species (Andow 1991; Landis and Marino 1999). This is especially true for annual monocultural cropping systems where the rates of establishment of imported natural enemies and their success in controlling the target pest are lower than in more stable and diversified cropping systems (Hall et al. 1980; Stiling 1990). Also, the systematic use of pesticides in crop production systems induced a limitation to the successful implementation of biological control. Pest control techniques have been notably developed to rely on improving cultural practices to minimize fertilizer and pesticide inputs (Gurr et al. 2004; Hassanali et al. 2008). Habitat management, a form of conservation biological control, is an ecologically based approach aimed at favoring natural enemies and enhancing biological control in agricultural systems (Fiedler et al. 2008; Landis et al. 2000). Numerous studies have shown that habitat management increasing structural diversity in agroecosystems resulted in a larger diversity of beneficials and often in less pest damages (Thies and Tscharntke 1999). This was particularly true with fields where intercropping (Fenández-Aparicio et al. 2007; Khan et al. 1997; Ma et al. 2007; Muhammad et al. 2012; Smith and McSorley 2000) and mixed cropping

(Ninkovic et al. 2011; Perrin and Phillips 1978; Schulthess et al. 2004; Tukahirwa and Coaker 1982) were applied. Field closed habitat management is an important element to develop sustainable agriculture by maximizing a range of ecosystem services that support crop production (Géneau et al. 2012).

Aphids (Homoptera: Aphididae: Aphidinae) are very abundant and destructive insect pests in agriculture, causing direct damages to plant crops but also as vectors for many important virus plant diseases (Kindlmann and Dixon 2010; Liu et al. 2009; Ma et al. 2010; Ng and Perry 2004). Regarding the next trophic level – the aphidophagous beneficials, aphids are attacked by a wide range of natural enemies (including predatory hoverflies, ladybirds and lacewings). Many of them have a high fecundity and are able to reduce aphid populations below the economic threshold. However, many predators are not very effective in locating aphid prey resulting from insufficient specific habitat requirements, such as the availability of a pollen or nectar supply as food for adults (Völkl et al. 2007).

Several plants as buffer strips in agroecological systems have been demonstrated to benefit for parasitoid wasps in the laboratory (Géneau et al. 2012; Nafziger Jr and Fadamiro 2011; Wäckers 2004; Winkler et al. 2009), hoverflies in cereal fields (Haenke et al. 2009; Hickman and Written 1996), blueberry fields (Walton and Isaacs 2011), cabbage fields

(White et al. 1995), and sweetcorn fields (Hickman and Wratten 1992). Relatively few plant-provided pollen, nectar, shelter and alternative hosts have been evaluated for use in habitat management and of those, just a few species have received the most attention (Fiedler et al. 2008). Broader views of the role of habitat management to not only enhance pest management but also to contribute increased ecosystem services may well contribute to the future of this important component of conservation biological control.

Association of plants, in mixture or in intercropping was also found to be potential crop field management to ensure lower colonization by pests and also higher biological control by occurring beneficials in plant multi-species combinations. The primary aims of the present investigation were to assess the effect of associating pea as buffer strips in wheat fields on the populations of aphids and their natural enemies. This change of wheat habitat was thought to be potential form of crop management to decrease the wheat attraction for pests and to provide alternative strategy for enhancing abundance of natural enemies and benefiting the conservation biological control.

2. Materials and methods

To assess the effect of wheat crop management on aphid pests and their natural enemy populations, field studies were conducted in a particular experimental design in the experimental fields of Gembloux Agro-Bio Tech, University of Liege, Namur Province of Belgium (50º33″N, 4º42″E) in 2011. Wheat (*Triticum aestivum* Linn) variety 'Tybalt' and pea (*Pisum sativum* Linn) variety 'James' were grown in our experiments.

Field experimental design

The field trial consisted of four treatments: (1) wheat mixed with pea (WMP), (2) wheat intercropped with pea (WIP), (3) wheat monoculture (WM), (4) pea monoculture (PM). A completely randomized positioned within wheat crops were settled by delimiting three distinct plots (4m × 10m each) for each treatment (total of 12 plots) (Fig. 1). Wheat monoculture was planted in 20-cm-apart rows at a rate of 350 seeds per m^2 on 18 February in 2011. Pea monoculture was planted in 50-cm-apart rows at a rate of 80 seeds per m^2 on 18 February in 2011. For wheat mixed with pea, pea was planted between the two rows of wheat at a rate of 35 seeds per m^2. No insecticides or herbicides were used in the whole experimental area. Wheat and pea were maintained with standard agronomic practices used in Europe.

Insect diversity and abundance monitoring

Coloured traps are frequently used to attract and catch insects (Laubertie et al. 2006). Yellow traps (26 cm diameter 10 cm depth) were attached to crabsticks and placed 10 cm above the surface of wheat plant. These traps were filled with water and a few drops detergent. 12 traps were placed in investigates plot (3 traps per treatment). Traps were emptied and reset at 7-day intervals between 4 May and 29 June. Trap contents were decanted through a 1-mm mesh sieve and transferred to 70% ethanol in plastic 50-mL vials. In the laboratory, aphids and their natural enemies were sorted and identified to the species level. Abundance of insects was recorded for each aphid and beneficial identified species.

Moreover, visual observations on plants were performed to compare with yellow trap methodology. Twenty crop tillers (pea and wheat, both when associated) were randomly selected in selected plots to visually assess the diversity and abundance of aphids on the tillers.

Statistical analysis

For all parametric tests, a data sqrt (n + 1) transformation was applied to stabilize the variance. The population densities of insects was compared among plot treatments using a one-way analysis of variance (ANOVA) (SAS 2001) followed by Fisher’s Least-Significant Difference s test

(LSD).

3. Results

Diversity of aphids and natural enemies according to kinds of wheat-pea association

Among the aphid recorded species, *M. dirhodum* and *S. avenae* were the predominant ones on wheat. On pea plants, *A. pisum* was the predominant species. PM and WM hosted the highest abundances of aphids with in traps and by visual observation investigation, respectively. Abundance of *A. pisum* was far higher than that of cereal aphids both by visual observations and trapping methods (Fig. 1 and Table1). In addition, several non-target aphid species were recorded in traps: *Cavariella aegopodii* (scopoli), *Aphis fabae* Scopoli, *Macrosiphum euphorbiae* (Thomas), *Myzus persicae* Sultzer, *Rhopalosiphum maidis* (Fitch), *Cavariella ihedbaldi*, *Nasonovia ribisnigri* (Mosley), *Phyllaphis fagi* L., *Chaitophorus* spp, *Capitophorus* spp.

Using yellow traps, the main aphid natural enemies were firstly the lacewings (46.1%), hoverflies secondly (43.1%) and ladybirds (10.8%). Among the natural enemy species recorded in the different treatments, *E. balteatus*, *C. carnea* and *H. axyridis* were the predominant species in investigated field plots (Table 1). Not all the collected hoverflies were aphidophagous species (*Eristalis pertinax, Helophilus trivitatus, Cheilosia spp, Eristalis tenax, Eristalis arbustorum*). We focused on aphid predator and their diversity was presented in Table 1. The highest abundances of aphidophagous species were observed in WIP and WMP plots, much more than in WM and PM as monospecies control plots.

Aphid abundance according to kinds of wheat-pea association

According to both visual observations and trapping, the population dynamics of *M. dirhodum*, *S. avenae* and *A. pisum* exhibited the same trends whatever the kind of plant association. The population densities of *M. dirhodum*, *S. avenae* and *A. pisum* reached their peak in all treatments on June 15th , June 22nd and June 22nd , respectively (Fig. 3 and 4).

Using visual observations within field plots, *M. dirhodum* was the most abundant in WM than in WIP and in WMP both on peak occurrence period and on whole experimental duration (Peak: $F_{2,6} = 37.90$, $P<0.01$; Total: $F_{2,6} = 20.44$, $P<0.01$). Similarly, a significant difference for *M. dirhodum* in trap was also detected among treatments (Peak: $F_{2,6} = 21.43$, $P<0.01$; Total: $F_{2,6} = 30.43$, $P<0.01$). Consistently with the results of *M. dirhodum*, the abundance of *S. avenae* by visual observations was higher in WM than in WIP and WMP both on peak occurrence period and on whole experimental duration (Peak: $F_{2,6} = 34.78$, $P<0.01$; Total: $F_{2,6} = 27.15$, $P<0.01$). Similar results were found for *S. avenae* using yellow traps (Peak: $F_{2,6} = 61.27$, $P<0.01$; Total: $F_{2,6} = 51.52$, $P<0.01$).

In addition, according to both trapping and visual observation investigations, the population density of *A. pisum* was reduced by mixing or intercropping wheat and pea (Fig. 4). The abundance of *A. pisum* was significantly lower in WIP and WMP than in PM (Trap: Peak, $F_{2,6} = 32.22$, $P<0.01$, Total: $F_{2,6} = 38.00$, $P<0.01$; Observation: Peak, $F_{2,6} = 31.38$, $P<0.01$; Total: $F_{2,6} = 79.64$, $P<0.01$).

Natural enemy abundance according to kinds of wheat-pea association

Lacewings reached their occurrence peak in all treatments on June 15th (Fig. 5A). The abundance of lacewings in each treatment was low before June 8th even if they were significantly more abundant in WIP than in others three treatments at that period ($F_{3,8} = 15.00$, $P<0.05$). The significantly higher abundance of lacewings was observed in WIP and WMP when comparing to monoculture crops ($F_{3,8} = 8.73$, $P<0.05$).

The population dynamic of hoverflies corresponded to an occurrence peak from 22nd to 29th of June (Fig. 5B). There was no significant difference in population density of hoverflies among treatments before peak period. After, the hoverfly densities in WIP and WMP were significantly higher than that in monoculture crops ($F_{3,8} = 114.43$, $P<0.05$). For total abundance, hoverflies significantly much more occurred in WIP followed by in WMP, in WM and PM (F3, 8 = 11.74, $P<0.05$).

A small fluctuation in population dynamic of ladybirds was observed according to the kinds of wheat and pea association in wheat growing season (Fig. 5 C). The total ladybird abundances in WIP and WMP were significantly higher than that ones in monoculture crops ($F_{3, 8}$ = 12.39, $P<0.05$).

4. Discussion

The aim of field habitat management is to create a suitable ecological infrastructure within the agricultural landscape to decrease pest pressure on crops and to provide resources such as food for natural enemies: alternative prey or hosts, and shelter from adverse conditions (Heimpel and Jervis 2005; Landis et al. 2000).

The abundance of lacewings, hoverflies and ladybirds in our investigation was improved by the presence of pea growing in wheat fields, indicating that those natural enemies prefer to select associating plant plots. Field study conducted by Haenke et al. (2009) also showed that hoverfly density and species richness of aphidophagous hoverflies were higher in narrow and broad sown flower strips compared to grassy strips and wheat-wheat boundary controls at the wheat peak-ripening stage. Similar results were also observed in blueberry fields (Walton and Isaacs 2011). Potential mechanisms of benefit for associating plant to natural enemies included the improvement of alternative food availability by providing habitat in which alternative hosts or prey are (Irvin and Hoddle 2007).

Further potential benefit of supplying pea plant in wheat field was suggested by the finding that the populations of cereal and pea aphids were both decreased obviously comparing to monoculture crops. Growing associating plant as “buffer strip” can be seen as dilution and decrease ways of plant availability for specialist pest species such as aphids but also as biodiversity increase in crop ecosystems. According to Root's natural enemies hypothesis, generalist and specialist natural enemies are expected to be more abundant in polycultures and therefore suppress herbivore population densities more in polycultures than in monocultures (Root 1973). The results obtained from the three aphid species, on the other hand, showed a high level of congruency with this hypothesis. In China, the maintenance of pea cover between rows of wheat crop reduced populations of insect pests *S. avenae* and enhanced the population and richness of natural enemies (Zhou et al. 2009a; Zhou et al. 2009b). Overall, the present results supported the hypothesis that provision of resources for natural enemies increases their abundance in adjacent crop fields without increasing the abundance of pest insects.

The rapidly expanding literature on habitat management is studied with attention to practices for favoring predators and parasitoids, implementation of habitat management to this developing area of conservation biological control. One of the major challenge, however, is the selection of plant species that encourage the population increase of beneficials while not encouraging the pest (Baggen and Gurr 1998). The selection criteria used to choose plants for habitat management research was reviewed by Fiedler et al (2008), that included: attractiveness to natural enemies, prolific production of pollen and/or nectar, accessibility of floral resources, flowering phenology, availability of seed, use of plants already present in, or adapted to, agricultural areas, previous success, and selectivity in favor of the natural enemy rather than its own natural enemies, or the pest itself.

Diversity in agro-ecosystems may favor reduced pest pressure and enhanced activity of natural enemies (Altieri et al. 1990; Altieri and Nicholls 2004). Overall, the above findings collectively suggest that there may be value in diversifying crop plant species in fields to reduce aphid populations and to increase aphidophagous beneficials by deploying plant mixing and intercropping in habitat management strategy. However, it has been shown that simply increasing diversity can exacerbate certain pest problems (Andow and Risch 1985; Baggen and Gurr 1998; Collins and

Johnson 1985; Moore 2010). Therefore, identifying the key elements of diversity may be a difficult process that can be guided by an understanding of the resources needed by natural enemies. The use of ‘selective food plants’ which allows only beneficial insects is proposed as an efficient and sustainable strategy in Integrated Pest Management.

5. Acknowledgements

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Figures and Tables

Figures

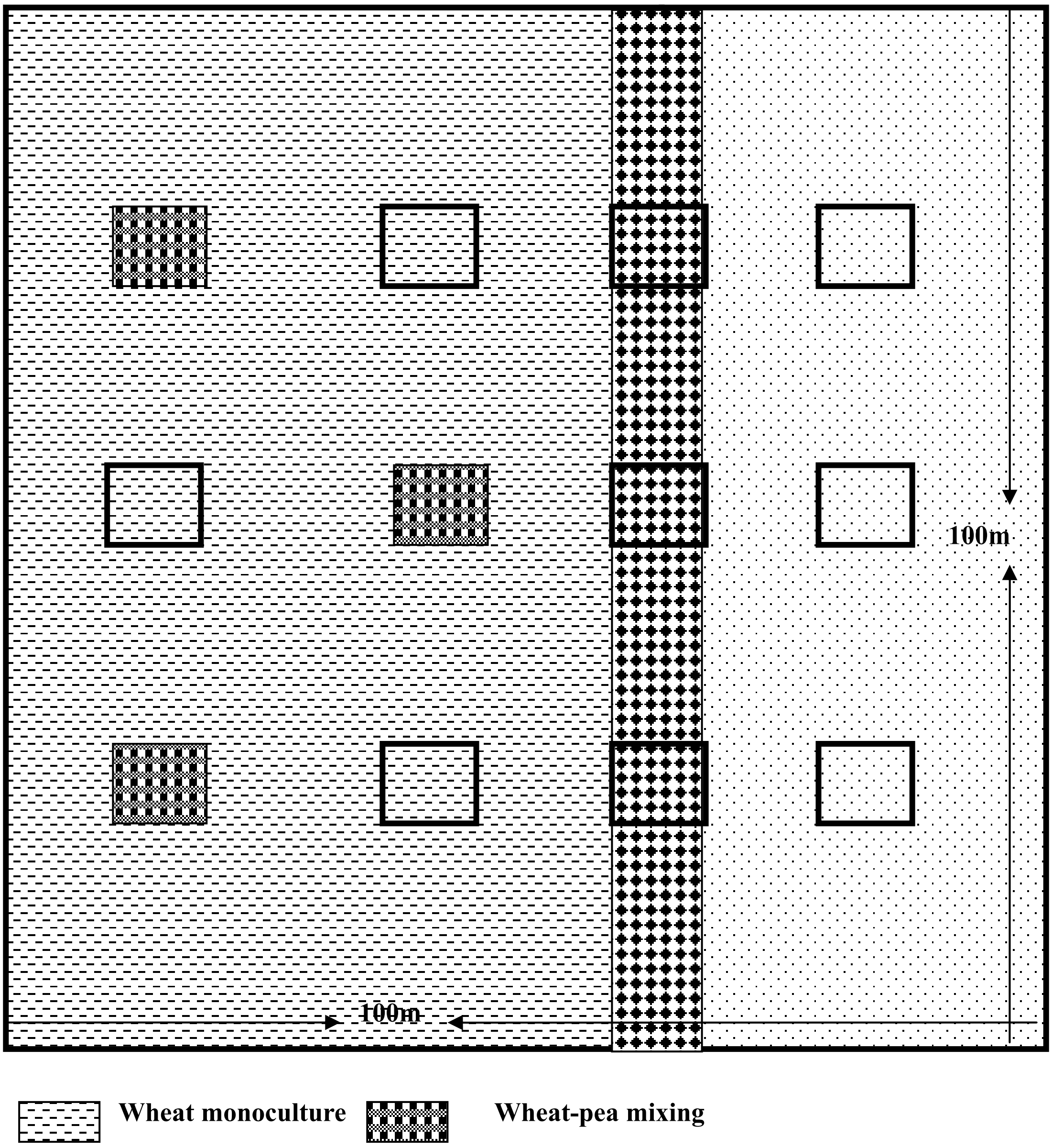


Fig. 1 The layout of experimental field.

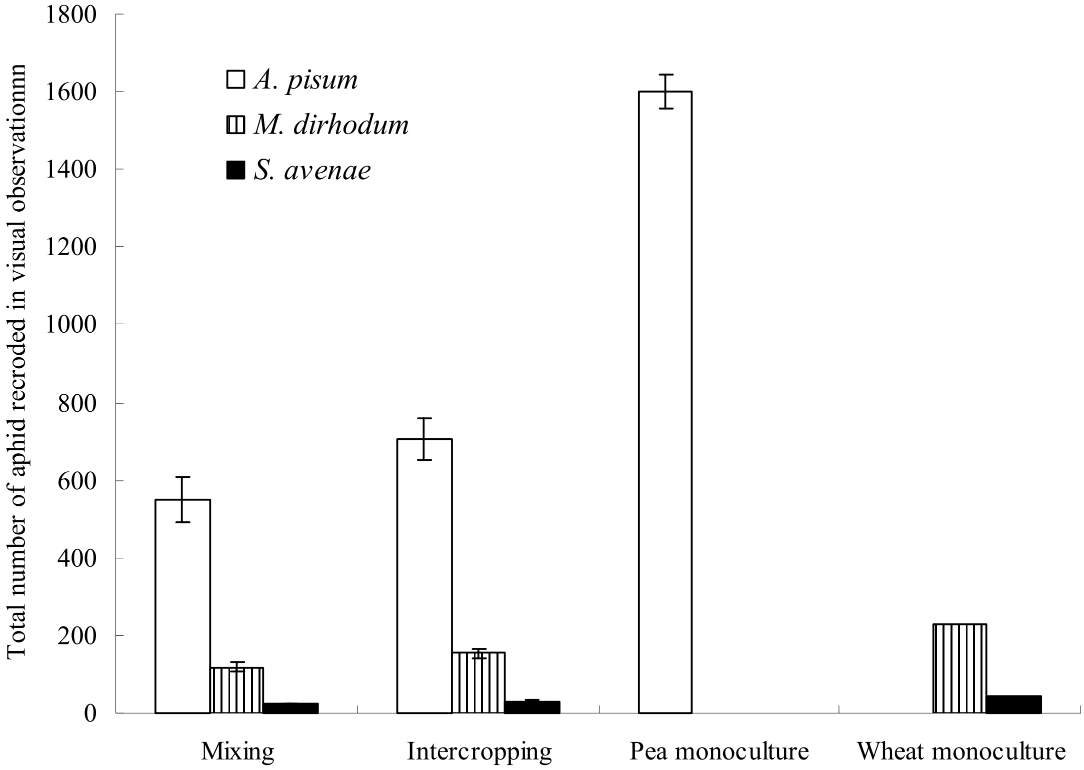
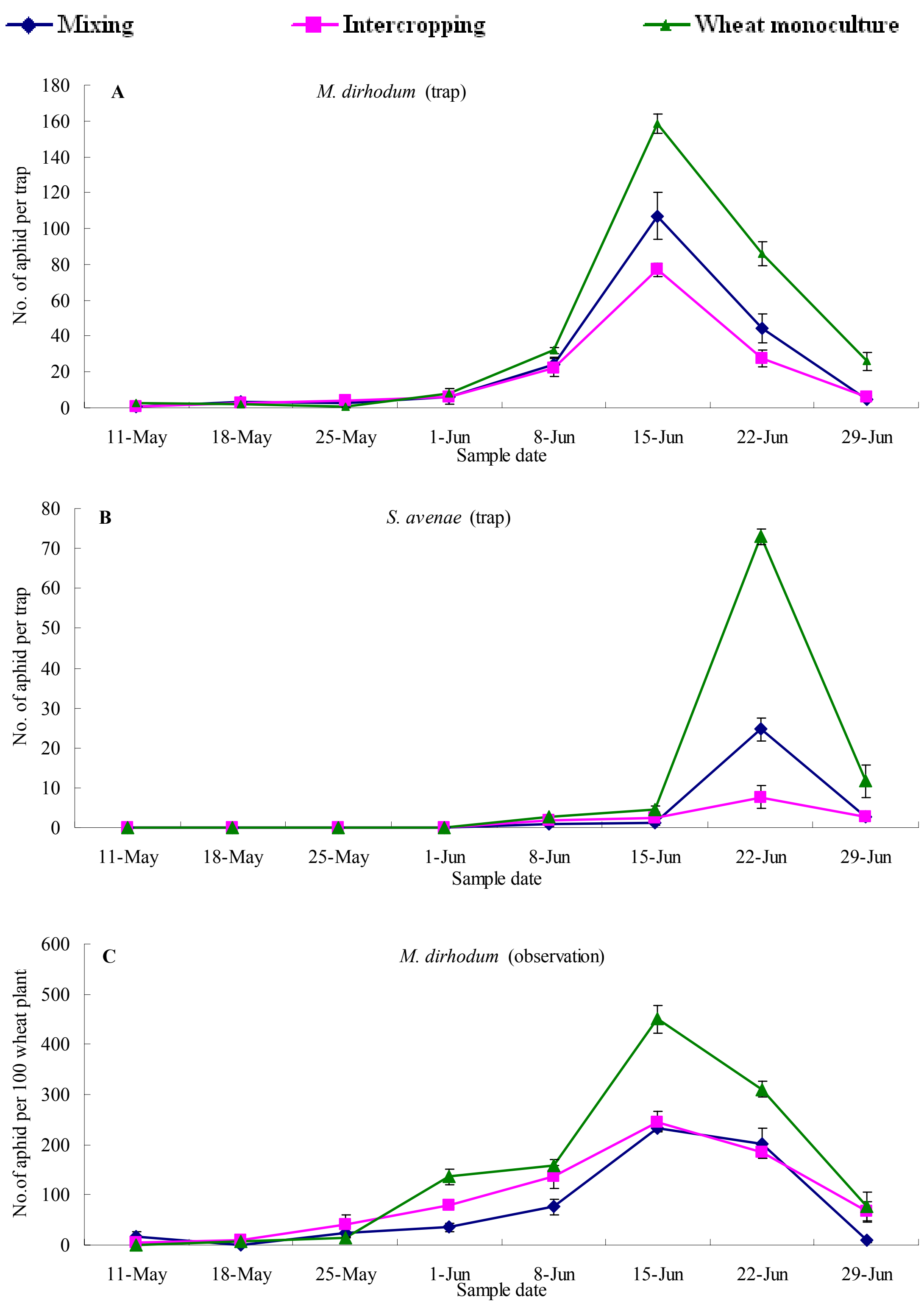


Fig. 2 Total number of aphid (Mean±SEM) recorded in visual observation according to kinds of wheat-pea association.


Mixing
Intercropping
Wheat monoculture
A
M. dirhodum (trap)
No. of aphid per trap
0
20
40
60
80
100
120
140
160
180
11-May
18-May
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
Sample date
B
S. avenae (trap)
No. of aphid per trap
0
10
20
30
40
50
60
70
80
11-May
18-May
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
Sample date
C
M. dirhodum (observation)
No.of aphid per 100 wheat plant
0
100
200
300
400
500
600
11-May
18-May
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
Sample date

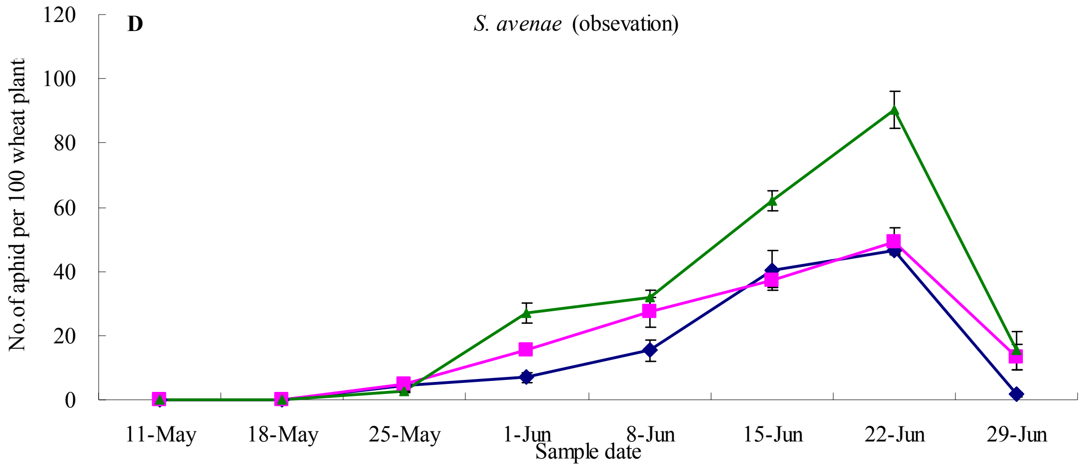


Fig. 3 Seasonal occurrence and abundance (Mean±SEM) of wheat aphids recorded according to kinds of wheat-pea association.

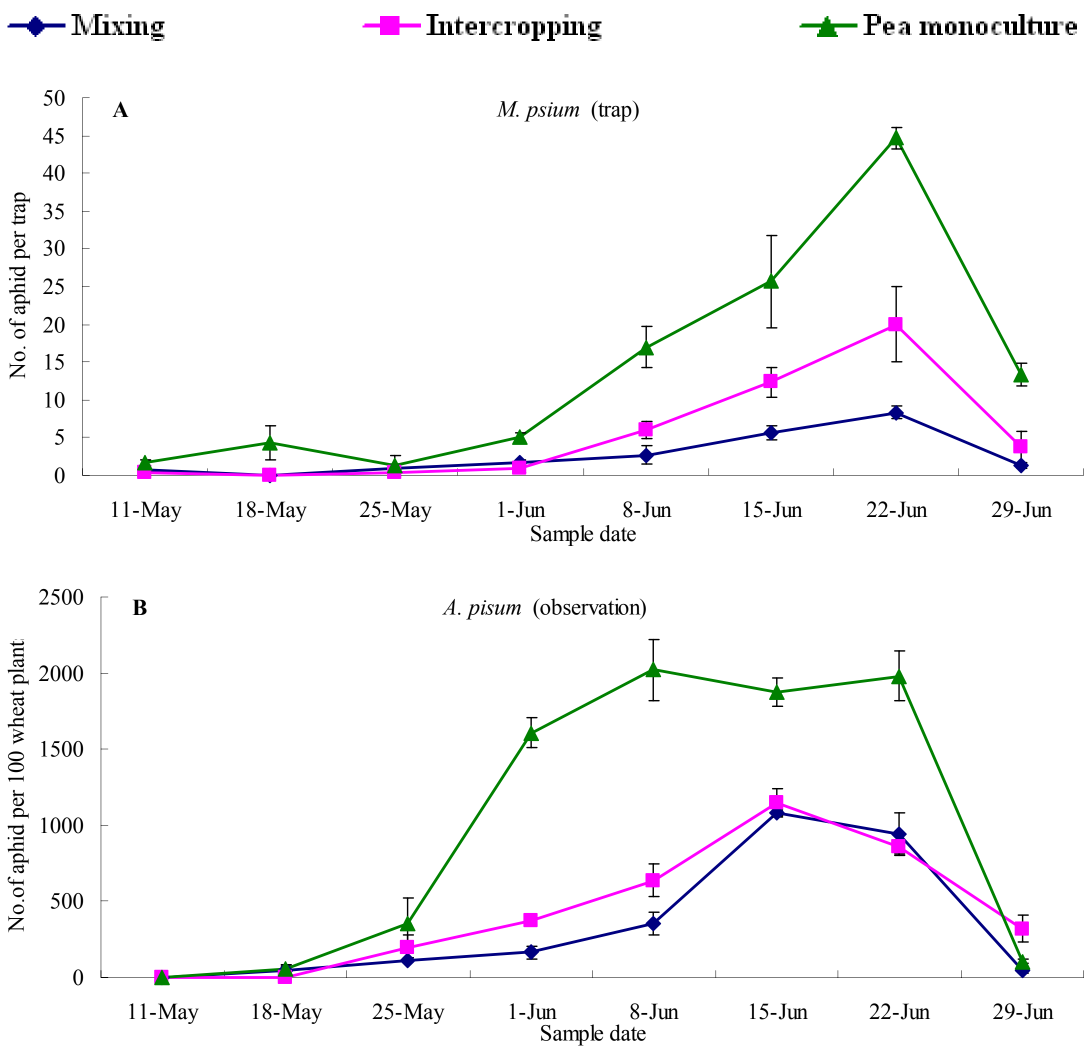


Fig. 4 Seasonal occurrence and abundance (Mean±SEM) of pea aphids recorded according to kinds of wheat-pea association.

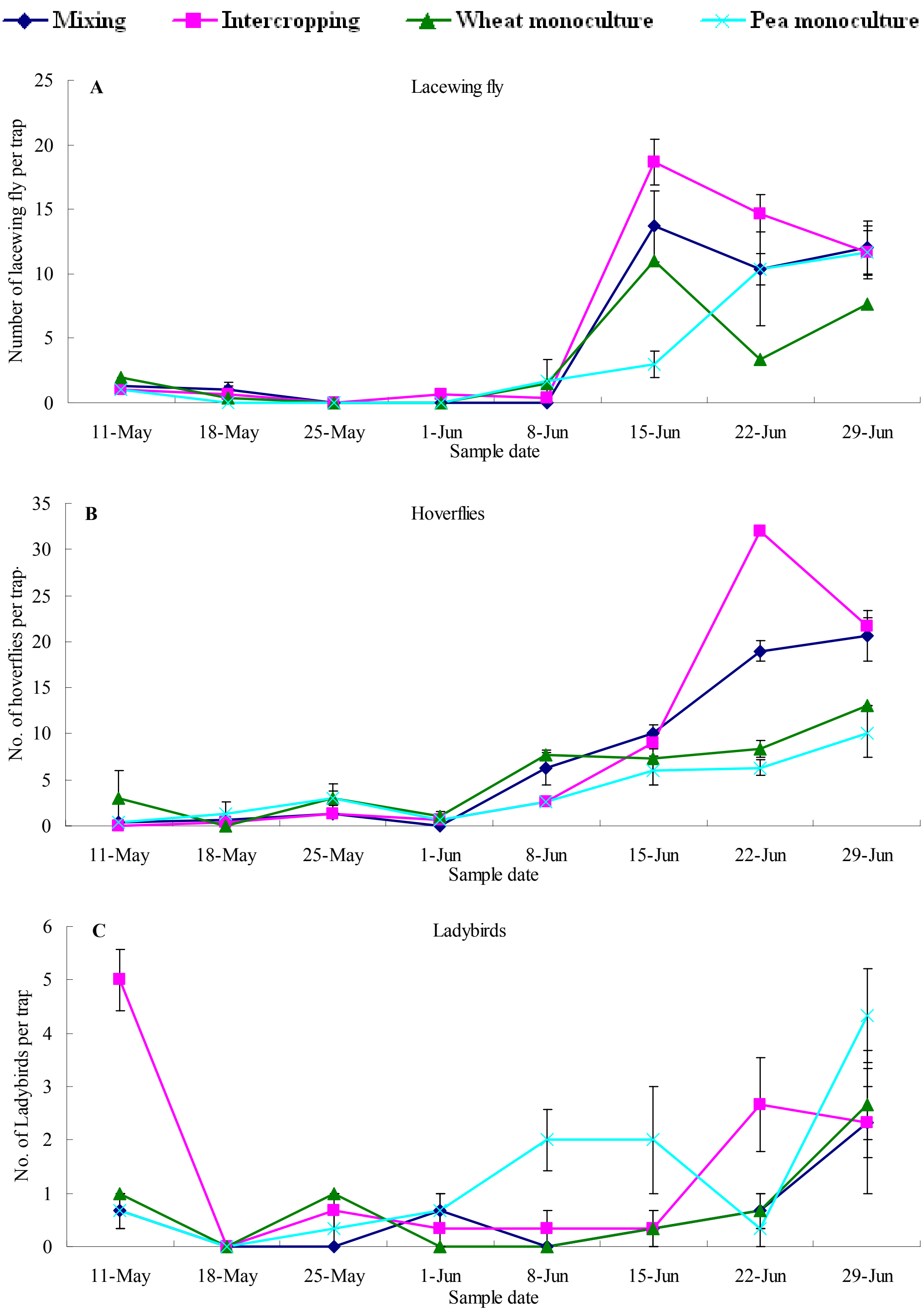


Fig. 5 Seasonal occurrence and abundance (Mean±SEM) of natural enemies recorded according to kinds of wheat-pea association.

Table 1. Total numbers of aphids and their natural enemies recorded in yellow traps in different crop systems throughout 2011 growing season

	Treatments				
Species	**wheat-pea mixing**	**wheat-pea intercropping**	**wheat monoculture**	**pea monoculture**	**%^{a}**
Aphids					
Metopolophum dirhodum (Walker)	578	437	949	0	67.58
Sitobion avenae (Fabricius)	89	43	276	0	14.04
Acyrthosiphon pisum Harris	64	131	0	339	18.38
Relative rates for each system **%**	**25.15**	**21.03**	**42.15**	**11.67**	
Ladybirds	10.83^b				
Coccinella septempunctata L.	5	17	8	9	40.21
Harmonia axyridis Pallas	5	14	8	18	46.39
Propylea 14-punctata	0	2	0	0	2.06
Harmonia 4-punctata	2	0	0	0	2.06
Calvia 14-guttata	1	1	1	4	7.22
Hippodamia variegata (Goeze)	1	1	0	0	2.06
Hoverflies	43.08^b				
Episyrphus balteatus De Geer	88	112	69	56	84.2
Scaeva pyrastri L.	0	3	2	0	1.3
Sphaerophoria scripta L.	5	8	4	0	4.4
Melanostoma scalare	0	1	2	0	0.78
Metasyrphus corollae	8	15	4	9	9.32
Lacewing fly	46.09^b				
Chrysoperla carnea Stephens	115	142	74	82	100
Total numbers of aphidophagous species	230	316	172	178	
Proportion of total numbers of	25.67	35.27	19.19	19.87	

aphidophagous species %

[a] Relative representation of each species by family

[b] Relative occurrence of each family in aphidophagous populations

Chapter VII: *GENERAL CONCLUSIONS, DISCUSSIONS AND PERSPECTIVES*

Chemical pesticides have been a boon all over the world, especially in developing countries in their efforts to eradicate insect-borne, endemic diseases, to produce adequate food and to protect crops. Controversy exists over the global dependence on such agents, given their excessive use or misuse, their volatility, long-distance transport and eventual environmental contamination in colder climates. Therefore, alternative stratehies of pest contol are desired relevant to maintain or improve crop`s productivity and sustainability.

Firstly, Our results showed that susceptible to wheat aphids was exhibited in most of the lines tested, and no immune and highly resistance lines to wheat aphids was observed. The average percentage of wheat germplasm lines with resistant, lowly susceptible, moderately susceptible and highly susceptible to aphid were 9.30%, 23.15%, 42.32%, and 25.23%, respectively. More importantly, 2 wheat germplasm lines (Lantian20, Lantian22) with the continuous resistance to wheat aphid in the five experimental stations over 2 years were discovered. It would be helpful to make wheat germplasm selections for breeding programs, especially if they have unique genes that may provide resistance to future biotypes of wheat aphids. It’s certainly the thing that plant breeders have not only sought to use host-plant resistance as a single-component control measure. A valuable method for evaluating the potential of aphid-resistance for wheat germplasm lines was also confirmed.

Secondly, Use of infochemicals to develop push-pull strategy in pest control is a potential way to promote sustainable crop production. (Z)-3-hexenol attracted aphids and should be considered as useful infochemical in aphid control by promoting attraction of aphids outside field plot. Releases of (E)-β-farnesene and garlic extraction allowed to significantly decrease the abundance of wheat aphids. The main natural enemies of cereal aphids were the lacewings (47.8%), the hoverflies (39.4%), and ladybirds (12.8%). Significant higher abundances of hoverflies and lacewings were found in Releases of (E)-β-farnesene and garlic extraction. Our results contribute to promote the "push-pull" strategy in aphid biological control based on releaser use with GE and EBF acting as pest pushing and beneficial pulling stimulus with Z3H for aphid pulling. Targeting the right volatiles for enhanced emission should lead to ecologically and economically sound ways of combating important pests. However, a remaining question surrounding the use of these materials in integrated pest management is to what are the ecological consequences of providing synthetic volatiles to predators and parasitoids in the absence of their prey. Therefore more detailed work on its ecological consequences, application rate, dose and duration under field conditions need to be done before those volatiles can be used to develop novel insect pest control strategies.

Finaly, Habitat management by crops intercropping or mixing, a form of conservation biological control, is an ecologically based approach aimed at favoring natural enemies and enhancing biological control in agricultural systems. Populations related to both crop species, *Metopolophum dirhodum* (Walker) and *Sitobion avenae* (Fabricius) in wheat but also *Acyrthosiphon pisum* Harris in pea obviously decreased when crop were grown in association. The high abundance of hoverflies, lacewings and ladybirds were found in wheat mixed with pea field, then in wheat intercropped with pea fields, more than in wheat and pea each alone. In addition, beneficial insect abundance in wheat-pea mixing or intercropping fields increased significantly in the latter half of the season. Our findings are discussed in relation to the use of combining plants as an alternative strategy in habitat crop management for efficient and sustainable pest control. Overall, the above findings collectively suggest that there may be value in diversifying crop plant species in fields to reduce aphid populations and to increase aphidophagous beneficials by deploying plant mixing and intercropping in habitat management strategy. However, it has been shown that simply increasing diversity can exacerbate certain pest problems. Therefore, identifying the key elements of diversity may be a difficult process that can be guided by an understanding of the resources needed by natural enemies. The use of ‘selective food plants’ which allows only beneficial insects is proposed as an efficient and sustainable strategy in Integrated Pest Management.

Indeed, according to study of this dissertation, we could partly and reasonably combine those strategies of host plant resistance, effective volatiles from plants and intercropping to regulate the abundance of cereal aphids and promote the stability of agricultural system.

Chapter VIII: *LIST OF PUBLICATIONS ORAL PRESENTATIONS AND POSTERS*

1. Publications

1 **Zhou Haibo**, Chen Julian, Liu Yong, Cheng Dengfa, Chen Lin, Sun Jingrui. (2009). The effect of using genetic diversity of wheat varieties for ecological regulation on *Sitobion avenae*. Acta Phytophyl Acica Sinica, 36(2): 151-156.

2 **Zhou Haibo**, Chen Julian, Cheng Dengfa, Liu Yong, Sun Jingrui. (2009). Efects of wheat-pea intercropping on *Sitobion avenae* and the functional groups of its main natural enemies. Acta Entomologica Sinica. 52(7):775-782.

3 **Zhou Haibo**, Chen Lin, Chen Julian, Liu Yong, Cheng Dengfa, Sun Jingrui. (2009). The effect of intercropping between wheat and pea on spatial distribution of *Sitobion avenae* based on GIS. Scientia Agricultura Sinica, 42（11）:3904-3913.

4 **Haibo Zhou**, Julian Chen, Dengfa Cheng, Frédéric Francis, Yong Liu, Jingrui Sun, Ying Huang, Xiaosong Wang, Xingwang Liu, Xiaoming Liu and Jiandong Zeng. Screening and identification of main germplasm resources and candidate cultivars in wheat resistance to aphids in China. African Journal of Biotechnology, 2011,10(63): 13930-13935.

5 **Zhou Haibo**, Chen Julian, Cheng Dengfa, Frederic Francis, Liu Yong, Sun Jingrui (2012). Effect of ecological regulation of biodiversity on insect in agroecosystems. Plant Protection, (1):6-10.

6 **Zhou Haibo**, Chen Julian, Cheng Dengfa, Liu Yong, Frederic Francis, Eric Haubruge, Claude Bragard, Sun Jingrui. (2011). Behavioral mechanism of combinations of wheat and pea on cereal aphid and natural enemies. The

annual conference of national plent protection, Beijing: China Agriculture Science and Technology Press, 812.

7 **Zhou Haibo**, Chen Julian, Liu Yong, Cheng Dengfa, Sun JR. (2008). Function and application of plant volatiles in ecological regulation and management of pest. The annual conference of national plent protection, Beijing: China Agriculture Science and Technology Press, 168-175.

8 **Zhou Haibo**, Chen Julian, Cheng Dengfa, Sun Jingrui, Zhang yunhui, Liu yong, Frederic Francis. (2009). Identification of volatiles from field wheat varieties of different resistance to aphids. The annual conference of national plent protection, Beijing: China Agriculture Science and Technology Press, 1036.

9 Xie Haicui, Chen Julian, Cheng Dengfa, **Zhou Haibo**, Sun Jingrui, Liu Yong, Frederic Francis. The function of ecological regulation to aphids in the wheat intercropping field. Plant Protection, (1):50-54.

10 Wang WL, Liu Y, Chen JL, Ji XL, **Zhou HB**, Wang G. (2009). Impact of intercropping aphid-resistant wheat cultivars with oilseed rape on wheat aphid (*Sitobion avenae*) and its natural enemies. Acta Ecologica Sinica, 29: 186-191.

11 Wang WL, Liu Y, Ji XL, Wang G, **Zhou HB**. (2008). Effects of wheat-oilseed rape or wheat-garlic intercropping on the population dynamics of *Sitobion avenae* and its main natural enemies. Chinese Journal of Applied Ecology, 19(6): 1331-1336.

12 **Haibo Zhou,** Julian Chen, Dengfa Cheng, Yong Liu, Claude Bragard, Eric Haubruge, Frédéric Francis. The overview of biodiversity conservation for the pest management in agroecosystems. Biotechnology, Agronomy, Society and Environment (under review)

13 **Haibo Zhou**, Julian Chen, Dengfa Cheng, Yong Liu, Claude Bragard, Eric Haubruge, Frédéric Francis. Adaptation of wheat-pea intercropping pattern in China to reduce *Sitobion avenae* (Hemiptera: Aphididae) occurrence by promoting natural enemies. Insect science.(under review)

14 **Haibo Zhou**, Julian Chen, Dengfa Cheng, Yong Liu, Claude Bragard, Eric Haubruge, Frédéric Francis. The influence of garlic intercropping or active emitted volatiles in releasers on aphid and related beneficial in wheat fields in China. Journal of Integrative Agriculture. (under review)

15 **Haibo Zhou**, Julian Chen, Dengfa Cheng, Yong Liu, Claude Bragard, Eric Haubruge, Frédéric Francis. Beneficial effect of associating pea to wheat: a laboratory approach on aphid and related predator behaviours. Biological control. (under review)

16 **Haibo Zhou**, Julian Chen, Dengfa Cheng, Frédéric Franciş, Yong Liu, Claude Bragard, Eric Haubruge. Influence of potato plant infection with Potato virus Y on the host preference of vectors: *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae). Arthropod-plant interaction (under review)

17 **Haibo Zhou**, Julian Chen, Yong Liu, Bernard Bodson, Eric Haubruge,

Dengfa Cheng, Frédéric Francis. Mixing crops and intercropping as efficient aphid control strategies: illustration with pea-wheat association. Journal of pest science (under review)

18 **Haibo Zhou**, Julian Chen, Yong Liu, Eric Haubruge, Dengfa Cheng, Frédéric Francis. Use of plant infochemical slow releasers to control aphids: a first investigation in Belgian wheat field. International journal of pest management (under review)

2. Oral Presentations

1 **Zhou haibo,** Chen Julian. Integrated cereal aphid management in China. Belgium Chinese scholars symposium, 10 July 2011, Namur, Belguim.

2 **Haibo Zhou**, Julian Chen, Dengfa Cheng, Yong Liu, Claude Bragard, Eric Haubruge, Frédéric Francis, Leroy Pascal, Farmakidis Julien.Effect of pea and wheat mixing: a laboratory approach on aphid and related predator behaviors. The 6th Asia-Pacific Conference on Chemical Ecology. Oct.11-15, 2011, Beijing, China.

3. Poster

1 **Hai-Bo Zhou**, Ju-Lian Chen, Deng-Fa Cheng, Yong Liu, Jing-Rui Sun. Efects of wheat-pea intercropping on *Sitobion avenae* and the functional groups of its main natural enemies. The 6th Asia-Pacific Congress of Entomology (APCE 2009), Beijing, China.18-22 Oct, 2009.

2 **Haibo Zhou**, Julian Chen, Yong Liu, Dengfa Cheng, Jingrui Sun.The effect of using genetic diversity of wheat varieties for ecological regulation on *Sitobion avenae.* The 6th Asia-Pacific Congress of Entomology (APCE 2009), Beijing, China.18-22 Oct, 2009.

3 **Haibo Zhou**, Julian Chen, Yong Liu, Dengfa Cheng, Claude Bragard, Eric

Haubruge, Frédéric Francis. Laboratory assessment of pea-wheat plant association coupled with potential aphid infestation on sitobion avenae behaviour：Hemipteran-Plant Interactions Symposium. Piracicaba,SP – Brazil. July 11-14, 2011.

4 **Haibo Zhou**, Julian Chen, Frédéric Francis , Dengfa Cheng, Yong Liu, Jingrui Sun.Evaluation for resistance to aphids of wheat germplasm resources in china. The 63rd International Symposium on Crop Protection, Gent, Belgium.24, May 2011.

5 **Haibo Zhou**, Julian Chen, Dengfa Cheng, Yong Liu, Claude Bragard, Eric Haubruge, Frédéric Francis. The influence of garlic intercropping, and related emitted volatiles, on pest incidence and yield in wheat fields. 14th Symposium on Insect-Plant Interactions, Wageningen, The Netherlands, 13-18 August 2011.