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# Intra- and intercrop diversification in cereal cropping and effect on pest control

Agathe Mansion-Vaquié

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# THESE DE DOCTORAT

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*par*

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**Diversité intra- et interspécifique dans les systèmes céréaliers et ses effets sur la régulation des ravageurs.**

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**Titre :** Diversité intra- et interspécifique dans les systèmes céréaliers et ses effets sur la régulation des ravageurs.

**Mots-clés :** Agroécologie; Régulation des ravageurs; Lutte biologique par conservation ; Mélanges variétaux; Couverts végétaux

**Résumé :**

Augmenter la diversité végétale au sein même du champ permet de réguler les populations de ravageurs dans de nombreux agroécosystèmes. Les mélanges variétaux (diversité intraspécifique) ou les associations de cultures avec une plante compagne (diversité interspécifique) sont considérées comme des pratiques agroécologiques prometteuses pour les systèmes de culture à bas intrants ou l'agriculture biologique. En effet, ces pratiques favorisent de nombreux services écosystémiques tels que la régulation des ravageurs, des maladies ou des adventices, ainsi que la fertilisation azotée. Cependant, le potentiel de régulation des ravageurs du blé par la combinaison de ces deux pratiques de diversification n'a pas encore été étudié.

Nous avons combiné ces deux pratiques dans le cadre d'expérimentations menées en plein champ et sur deux saisons de culture, afin d'examiner leurs impacts sur les populations de pucerons et d'ennemis naturels. Nous avons également évalué le potentiel de régulation des ravageurs en mesurant les taux de prédation de proies sentinelles.

La combinaison des diversités intra- et interspécifique n'est pas plus performante pour réduire les populations de pucerons que les pratiques prises séparément. L'association de culture blé-trèfle tend à être moins infestée par les pucerons, tandis que le mélange variétal est plus infesté que la variété la moins sensible. Les variations annuelles des conditions climatiques impactent fortement le développement du blé et du trèfle, ainsi que la date d'apparition du pic de puceron. Le rendement du blé, ainsi que le taux d'azote du grain sont réduits par l'association de culture par 7 à 10%, mais pas par le mélange variétal. La présence d'un couvert de trèfle dans les champs de blé, semble avoir favorisé la biodiversité fonctionnelle, particulièrement les ennemis naturels tels que les carabes, mais pas le mélange variétal. Les résultats sont variables selon la famille d'arthropodes concernée et leur position au sein du couvert végétal (au sol ou dans le feuillage). Le couvert de trèfle et le champ ont influencé la composition de la communauté de carabes prédateurs. Les taux de prédation des proies sentinelles n'ont pas été impactés par les pratiques de diversifications.

En laboratoire, nous avons évalué comment l'association du blé avec des légumineuses (trèfle ou pois) pouvait modifier le comportement du puceron du blé *Sitobion avenae* en terme de location de sa plante hôte et du développement de la population. Les pucerons ont résidé moins de temps sur le blé quand il était associé à du trèfle. Les populations de pucerons se sont moins développées dans les associations du blé avec une légumineuse par rapport à du blé seul, mais si l'on prend en compte la biomasse du blé, seulement l'association blé-trèfle a considérablement réduit les densités de pucerons sur le blé. Ainsi l'espèce associée et sa densité sont des paramètres importants qui devraient être pris en compte dans les études sur la diversité interspécifique, car ils pourraient expliquer la grande variation dans les résultats rapportés par les analyses bibliographiques.

Nos résultats suggèrent qu'augmenter la diversité cultivée au sein du champ peut aider à réguler les pucerons dans une certaine mesure, mais la combinaison des deux pratiques de diversification ne résultent pas en un trade-off entre la régulation des ravageurs et les performances agronomiques particulièrement attractifs pour les agriculteurs.

**Title :** Intra- and intercrop diversification in cereal cropping and effect on pest control

**Keywords :** Agroecology; Pest control; Conservation Biological Control ; Variety mixtures;  
Cover crop

**Abstract :**

Increasing intrafield plant diversity has been shown to regulate pest populations in various agroecosystems. Polyvarietal mixtures of a crop species (intraspecific diversity) or associations of a crop and a companion plant (interspecific diversity) are both considered as promising agroecological practices for low-input or organic agriculture systems by providing several ecosystem services such as pest, disease and weed control, and nitrogen fertilization. However, combining both diversification practices has not been studied yet in perspective of winter wheat pest control.

In organic field experiments over two growing seasons, we combined both practices and examined the direct impact on aphid and natural enemy populations and on wheat production. We also investigated the potential pest regulation service through the assessment of the rate of predation by using sentinel preys.

Results show that combining intra- and interspecific diversity did not outperform each practice individually in reducing aphid populations, thus not clearly showing synergetic effects. Taken separately, intercropping tended to have lower aphid infestation, while the cultivar mixtures was more infested by aphids than the least susceptible cultivar. Yearly variation in climatic conditions strongly impacted wheat and clover development, as well as the appearance of aphid peaks. Wheat yields and grain nitrogen content were reduced in intercropping by 7 to 10%, but not in cultivar mixtures. Functional biodiversity, especially natural enemies such as ground beetles, tended to be positively correlated to the presence of a clover cover in the wheat fields (interspecific diversification), but did not respond to the wheat cultivar mixture (intraspecific diversification). Results varied according to the family of arthropods concerned and their position within the vegetation layer (ground dwelling or foliage dwelling arthropods). The cover of white clover and the field context influenced the community composition of predatory ground dwelling beetles. Rates of predation on sentinel preys were not influenced by any of the diversification practices.

Under laboratory conditions, we evaluated how combining wheat and legumes (clover or pea) modifies the behaviour of the cereal aphid *Sitobion avenae* in terms of host-plant location, and population growth. We observed that aphids' residence time on wheat was decreased when this host-plant was intercropped with clover. At the population level, wheat-legume intercrops reduced the number of aphids on wheat plants compared to wheat sole crops but if we take into account plant biomass, only intercropping clover with wheat significantly reduced aphid densities on wheat. The species used as non-host plants and their density are important parameters that should be taken into account in studies on intercropping systems and that may explain the large variability in the results observed in the literature.

Our findings suggest that intrafield diversification may regulate wheat aphids to some extent, but combining the two diversification practices did not result in an interesting trade-off between pest regulation and wheat production in real farming conditions.

## **Avant-propos**

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Ce travail de thèse a été effectué au sein de l'unité de recherche Agroécologie et Environnement de l'Institut Supérieur d'Agriculture Rhône-Alpes (ISARA) à Lyon sous la direction d'Alexander Wezel et l'encadrement d'Aurélié Ferrer. Les différentes expérimentations ont été menées dans le cadre du projet Biodiv+ financé par la Compagnie Nationale du Rhône.

Ce programme souhaite participer de façon active et opérationnelle à la réduction des impacts négatifs des activités agricoles sur l'environnement en intervenant notamment sur la biodiversité et la qualité de l'eau en Boucle du Rhône. Ce programme devrait permettre de proposer aux agriculteurs des pratiques culturales scientifiquement renseignées, techniquement réalistes qui leur permettraient d'améliorer le contrôle naturel des populations de ravageurs et des maladies et donc de diminuer de façon sensible leur utilisation de pesticides.

Différents travaux scientifiques, publications et communications sont directement issus de ce travail de thèse.

### **Articles scientifiques**

**Mansion-Vaquié A.**, Wezel A. & Ferrer A. (2018). Wheat genotypic diversity and intercropping to control cereal aphids. *Agriculture, Ecosystems and Environment* (under review)

**Mansion-Vaquié A.**, Ferrer A., Ramon-Portugal F., Wezel A. & Magro A. (2018). Intercropping impacts the host location behaviour and population growth of aphids (Hemiptera: Aphididae). *Entomologia Experimentalis et Applicata* (under review)

**Mansion-Vaquié A.**, Tolon V., Wezel A. & Ferrer A. (2019). Combining intraspecific and interspecific crop diversification for improved conservation biological control in wheat fields. *Agricultural and Forest Entomology* (to be submitted)

### **Communications orales**

**Mansion-Vaquié A.** & Ferrer A. (2018). Stacking crop varieties and intercropping: impacts on aphids in wheat fields. In: *XI European Congress of Entomology*, 2-6<sup>th</sup> of July 2018, Napoli, Italy.

Ferrer A., Tolon V. & **Mansion-Vaquié A.** (2018). Multilevel plant diversity for enhanced conservation biological control and crop system resilience. In: *XI European Congress of Entomology*, 2-6<sup>th</sup> of July 2018, Napoli, Italy.

### **Posters**

**Mansion-Vaquié A.**, Lascoste M., Ferrer A. & Wezel A. (2017) Intercropping winter wheat and white clover to enhance beneficial ground beetles. In: *18th European Carabidologist Meeting*, 25-29<sup>th</sup> of September 2017, Rennes, France.

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---

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## **Chapter 1:**

### Introduction and research questions

Over the last centuries, plant production efficiency increased significantly thanks to improved fertilisation, plant breeding and plant protection, leading to higher yields. However, cultivated plants have always been and will continue to be damaged by many pests which reduce their productivity (Oerke, 2005), in particular if cropped in monocultures on larger areas (Wetzel et al., 2016). The challenge of regulating pest populations has been addressed in the dominant conventional agricultural model through the massive use of synthetic chemical inputs (e.g. pesticides, herbicides). While these methods have unquestionably increased yields at a global scale (Tilman et al., 2002), the rise in crop productivity was accompanied by negative externalities with high costs for human health and for the environment (Pimentel, 2005; Mostafalou & Abdollahi, 2013; Annett et al., 2014; Gibbons et al., 2015). Especially, populations of beneficial arthropods that are biological control agents or pollinators, and provide different ecosystem services are declining also due to pesticide use (Geiger et al., 2010; Potts et al., 2010; Oliver et al., 2015). Counterproductive effects even appeared with records of pest resurgences due to the elimination of their natural enemies and/or the development of resistant pest (Hill et al., 2017).

Additionally, agricultural intensification over the last decades was accompanied by a considerable decline in intrafield heterogeneity through the use of techniques that standardize the management of crops spatially and temporally (Benton et al., 2003). Especially the development and adoption of modern cultivars of crops has increased considerably, accompanied generally by an important loss in genetic diversity (FAO, 1997; Wouw et al., 2009; Tooker & Frank, 2012). Cereals crops, especially wheat, rice and corn, represent dominant crops worldwide, providing 60% of human food (Tilman et al., 2002). The demand for wheat is projected to increase greatly in the “developing” world, where modern cultivars reach about 90% of the area in these regions (Shiferaw et al., 2013). For both durum and bread wheat varieties, the spread of such modern cultivars results in an overall decrease of genetic diversity and appears to be associated with loss of some quality traits (Newton et al.,

2010). Genetic erosion (i.e. the loss of variation in crops) may also occur at the species level, though it has not been clearly demonstrated yet for crop species (Wouw et al., 2009). Agricultural intensification and increased density of uniform cereal crops resulted also in loss of weed species diversity (Weiner et al., 2001; Fried et al., 2009; Arslan, 2018) with potential cascading effects on arthropods (Norris et al., 2000).

Overall, the reliance on pesticides and the homogenization of the cropped fields have negative impacts on beneficial arthropods and especially the natural enemies that may regulate the pests in agroecosystems. Within this context, a major concern is to develop more sustainable practices regarding the agricultural production systems, especially in term of reduction in the use of pesticides in order to decrease their impact on human health and the environment (Tilman et al., 2002).

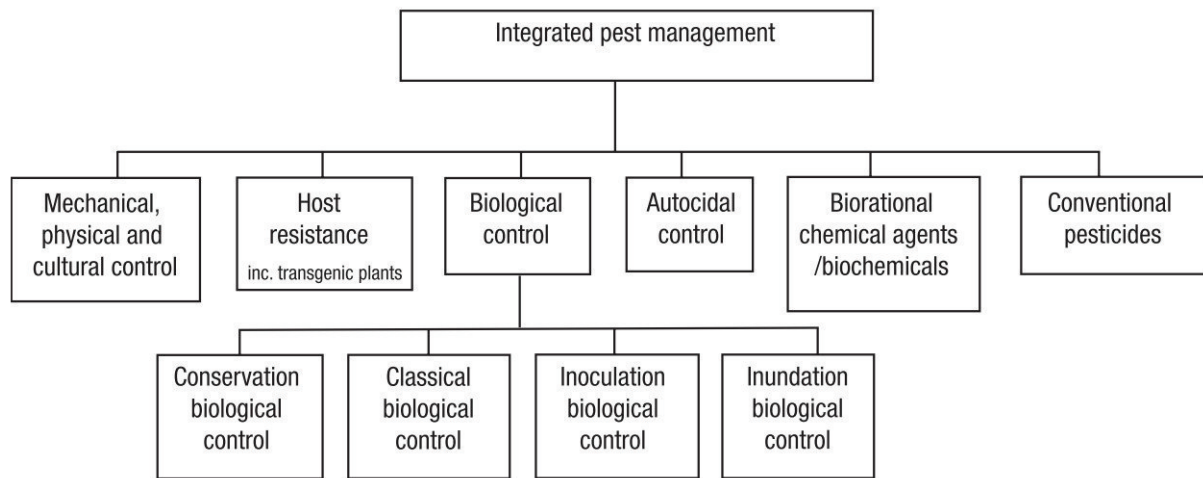
## **1.1 Alternative to pesticides: the development of systemic approaches**

To meet this challenge different approaches, developed in the last decades, aim at reducing pests through a better understanding of the ecological processes involved and the use of alternative methods to pesticides.

### **1.1.1 Integrated pest management**

As an alternative to the conventional and sole use of pesticides, different methods and practices exist today to regulate pests. Most of those methods are gathered under the Integrated Pest Management (IPM) approach (Figure 1.1). IPM tends to switch crop protection systems from a broad use of pesticides towards specific management tools that are complementary and based on natural regulatory mechanisms such as biological control approaches or improved host-plant resistance through genetic selections of crops (Pedigo & Buntin, 1993; Eilenberg et al., 2001; Gurr et al., 2004). Biological control is defined as "the

use of living organisms to suppress the population density or impact a specific pest organism, making it less abundant or less damaging than it would otherwise be" (Eilenberg et al., 2001).



**Figure 1.1: Methodological and practical approaches used in IPM (reproduced from Eilenberg *et al.*, 2001).**

In addition, cultural control involves the modification of how to grow the crop in order to reduce the pest fitness (e.g. crop rotation, planting dates) (Wratten et al., 2007). Other methods include behavioral control (e.g. pheromones, repellent) and physical control (e.g. traps or mulches) (Norris et al., 2003). Within IPM, the pests can still be controlled by conventional pesticides, but their use is considerably reduced due to the complementary adoption of alternative methods (Pretty, 2005). IPM requires a sound understanding of pest and crop ecology and a holistic ecological approach to the agroecosystem which should help to design cultivation systems less vulnerable to pest outbreaks and one that works against the pests' performance (Norris et al., 2003; Gurr et al., 2004).

### 1.1.2 Agroecological practices

Agroecology proposes to go a step further in this systemic approach adopted by IPM, in keeping therapeutic methods (e.g. conventional pesticides, biopesticides or biological control agents) only as backups while developing the ability of the cropping system to inherently regulate pests (Lewis et al., 1997; Nicholls & Altieri, 2004; Birch et al., 2011). The set of practices promoted under the umbrella of Agroecology, so called *Agroecological practices*,

are "agricultural practices aiming to produce significant amounts of food, which valorise in the best way ecological processes and ecosystem services in integrating them as fundamental elements in the development of the practices" (Wezel et al., 2014). This ambition to rely on natural ecological processes and to enhance ecosystem services in agroecosystems can range from a simple substitution of a given practice by another more sustainable, to a full redesign the cropping systems with deep implication on the system management by the farmer (Hill & MacRae, 1995). To achieve both production and balanced pest–natural enemy populations agroecological approach generally banks on a diversification of the cropping system at different scales: at a field scale through an increasing of the diversity of cultivars, crops, crop rotation, and/or at a larger scale with consideration and management of the semi-natural elements surrounding the field or even of the landscape matrix (Wezel *et al.*, 2014).

### 1.1.3 Habitat manipulation

Within both IPM and agroecological approaches, several practices include the manipulation of the biological environment of the crop in order to regulate pests and especially the manipulation of the vegetation such as mulch, intercropping, and non-crop habitat (Landis et al., 2005). In this case, we talk more precisely of habitat management or manipulation, defined as "an intervention in an agroecosystem's vegetation with the intended consequence of suppressing pest densities" (Gurr et al., 2017).

Habitat manipulation may suppress pests through cultural control, which covers a wide range of agronomic practices and techniques that influence directly or indirectly the behaviour of pests towards their host plant (Figure 1.2); or through conservation biological control, defined as "the modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce pests" (Eilenberg et al., 2001) (Figure 1.3). Most of habitat manipulations rely on the increase in vegetation diversity at the field scale and eventually in the surrounding landscapes.

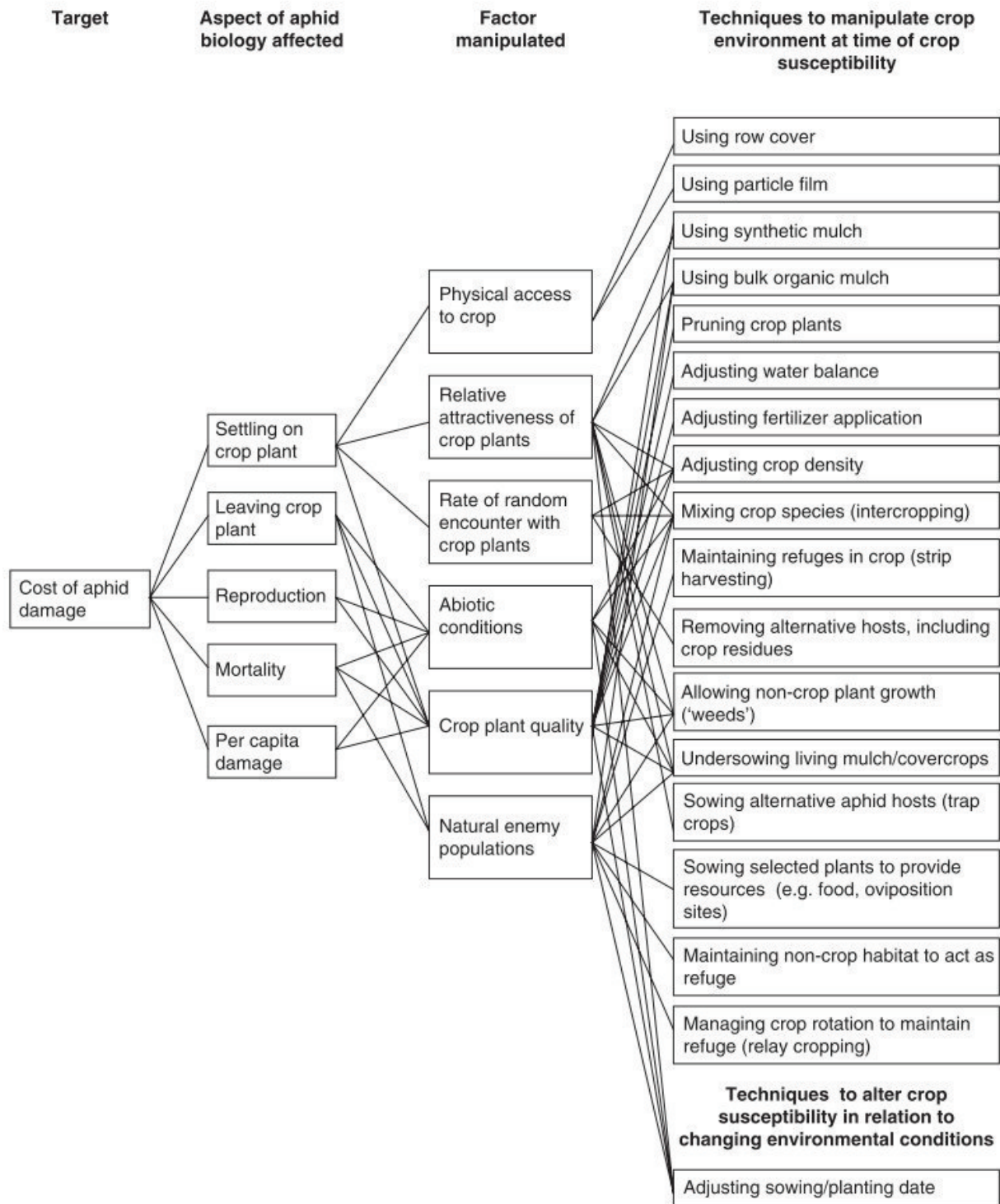
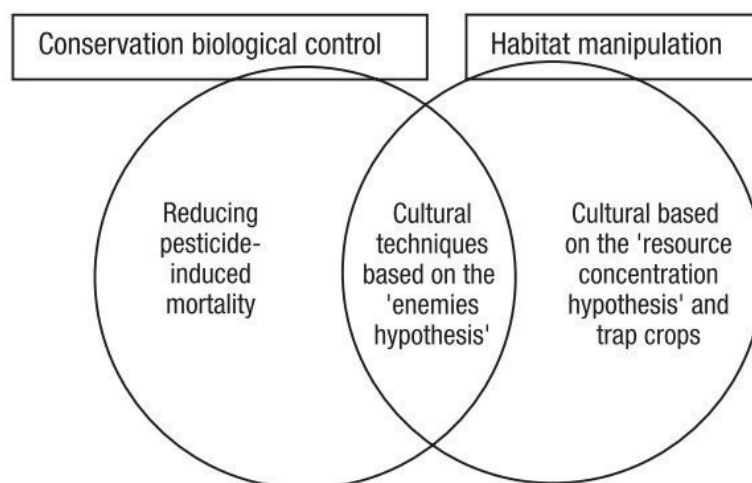


Figure 1.2: Cultural practices and techniques for managing aphid populations and the mechanisms by which they affect levels of aphid damage (reproduced from Wratten et al., 2007).



**Figure 1.3: Comparing and contrasting habitat manipulation and conservation biological control approaches to pest management (reproduced from Gurr et al., 2004).**

## **1.2 Diversifying the vegetation to control pests**

The idea that diversification may benefit the regulation of pest within the agroecosystem (which is found either in IPM, agroecology or habitat manipulation approaches) relies on the presence of different ecological mechanisms at work in agroecosystems. Diversification may be implemented through different practices at different scales.

### **1.2.1 Bottom-up and top down regulation of pests**

Increasing plant diversity has been shown to regulate pest populations in various agroecosystems (Hooks & Johnson, 2003; Letourneau et al., 2011; Dassou & Tixier, 2016). This refers to the associational resistance phenomenon (Tahvanainen & Root, 1972), which can be explained by two ecological processes: bottom-up control occurring when the herbivores are regulated by the lower trophic level (crops and non-crop plants) and top-down control occurring when the natural enemies regulate the herbivores (Gurr et al., 2004). According to the resource concentration hypothesis (Root, 1973), specialized herbivores are more likely to find and remain on concentrated host plants. Polycultures are therefore less

favorable environments than monocultures due to a dilution effect of the host plants by altering profile odor or the visual stimuli of the host plant (Andow, 1991; Poveda et al., 2008; Malézieux et al., 2009). Moreover, the association of different species or varieties of crops may modify the vegetation structure (the barrier crop hypothesis) that may hamper the movement of the herbivores (Poveda et al., 2008; Malézieux et al., 2009). In addition, natural enemies are expected to be more diverse and abundant in such complex environments (natural enemy hypothesis; Root, 1973) due to the provision of shelter, nectar, alternative prey/hosts, and pollen, promoting the presence of natural enemies (Gurr et al., 2017). These regulation processes are therefore not mutually exclusive and diversification practices at any scales may favor both (Gurr et al., 2004).

### 1.2.2 Diversifying around the field

Around the field, habitat management practices involve the manipulation of the vegetation from the field borders towards the landscape composition and configuration (Figure 1.4). Field margins are located between the crop and the boundary and composed of grass or flowers that enhance the vegetation diversity in terms of species and structure (Barbosa, 1998; Marshall & Moonen, 2002). Field margins act as a source of biological control agents towards the crops (Hawthorne et al., 1998; Denys et al., 2002), which may consequently enhance pest control in the fields (Holland et al., 2008; Balzan & Moonen, 2014). Management of semi-natural landscape elements such as hedgerows, woodlands or vegetation strips including buffer strips and beetle banks, may also support natural enemies and enhance their ability to regulate pest in the fields (Holland et al., 2016). The landscape composition and configuration are also influencing natural enemies and pests, and might be managed in order to optimize conservation biological control (Bianchi et al., 2006; Veres et al., 2013; Martin et al., 2015).



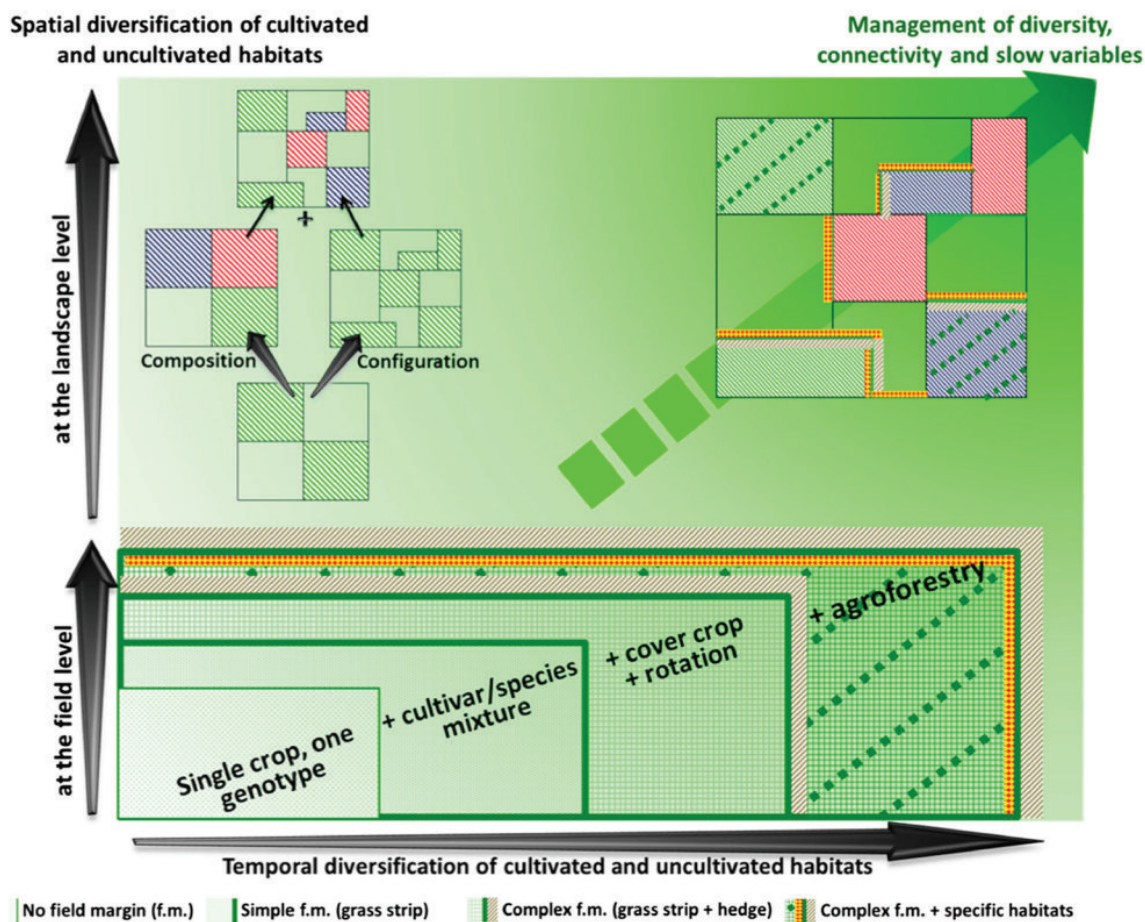


Figure 1.4: The increase in plant diversity in time (X-axis) and in space (Y-axis) at field, field margin, and landscape levels (reproduced from Duru et al., 2015).

There are however several limits to the implementation of habitat manipulation at this scale by farmers. They do not see the benefits, especially from an economic point of view, that such manipulation may offer and cost-benefits analyses of conservation biological control measures are lacking (Cullen et al., 2008). Farmers often consider the management of semi-natural elements as a waste of potential cropland and barriers for mechanization (Tschardt et al., 2016). This feeling is reinforced by the important variability in the effectiveness of conservation biological control measures (Tschardt et al., 2016; Begg et al., 2017). Landscape elements are even seen as a source of pests by farmers in orchards rather than benefits (Salliou & Barnaud, 2017).

### 1.2.3 Diversifying at the field scale

At the field scale, farmers may typically manage the *planned biodiversity*, which refers to the diversity of cash crops, forage or cover crops at the species or cultivar levels that are

intentionally chosen by the farmer and their spatial and temporal layouts as determined in the crop rotation (Figure 1.4; Duru *et al.*, 2015). Increasing plant diversity and especially the planned biodiversity at the field scale is of particular interest for farmers, because such agroecological practices rely on the optimization of the ecological processes within the cultivated area (Iverson *et al.*, 2014; Brooker *et al.*, 2015; Garibaldi *et al.*, 2017). This means that farmers do not have to lose a part of their arable area to implement vegetation in order to control pests and/or attract natural enemies or pollinators, which can be seen as a constraint for them (Landis *et al.*, 2000; Gurr *et al.*, 2017). Additionally, reliance on natural enemies to control pests are too uncertain and may discourage farmers to drop pesticides for investing into complex and time-consuming management practices (Dedryver *et al.*, 2010). The delivery of a panel of ecosystem services is therefore a key element to convince farmers to adopt practices based on habitat manipulation in order to control pest (Gurr *et al.*, 2017). Therefore, practices that increase the planned biodiversity at the field scale and that are known to deliver multiple ecosystem services, besides pest control, have a good potential to be implemented by farmers.

### **1.3 Intrafield diversification practices to promote pest regulation and other associated ecosystem services in cereal cropping systems**

At the field scale, Andow (1991) distinguishes three components of the vegetational diversity: the kinds, the spatial array, and the temporal overlap of the plants (Figure 1.5). The kinds refer to which plant is combined together. We differentiate intraspecific and interspecific diversification that concerns the increase of diversity at the genetic and at the species level respectively. Both intraspecific and interspecific practices are presented in more details at section 1.3.1 and 1.3.2 respectively. Diversification practices can be distinguished according to the spatial arrangement of the associated plants. Andrews & Kassam (1976) categorised intercropping into four types based on the spatial and temporal overlap of plant species:

mixed intercropping - no distinct row arrangement, row intercropping – plants are grown in separate alternate rows, strip intercropping – plants are grown in alternate group of rows, and relay intercropping – the second crop is sown during the growth of the first crop. Mixed intercropping can also refer to interspersed diversification, while aggregated diversification refers to row or strip intercropping because they imply a certain degree of spatial separation between plant types (Sunderland & Samu, 2000). We can further distinguish between additive - addition of both densities of plants compared to monoculture, and substitutive designs - total density equals the monoculture, so the density of each single species is reduced (Malézieux et al., 2009). Finally, temporal overlap of the different plant species may vary from none as in crop rotation, intermediate as in relay intercropping or complete as in simultaneous intercropping.

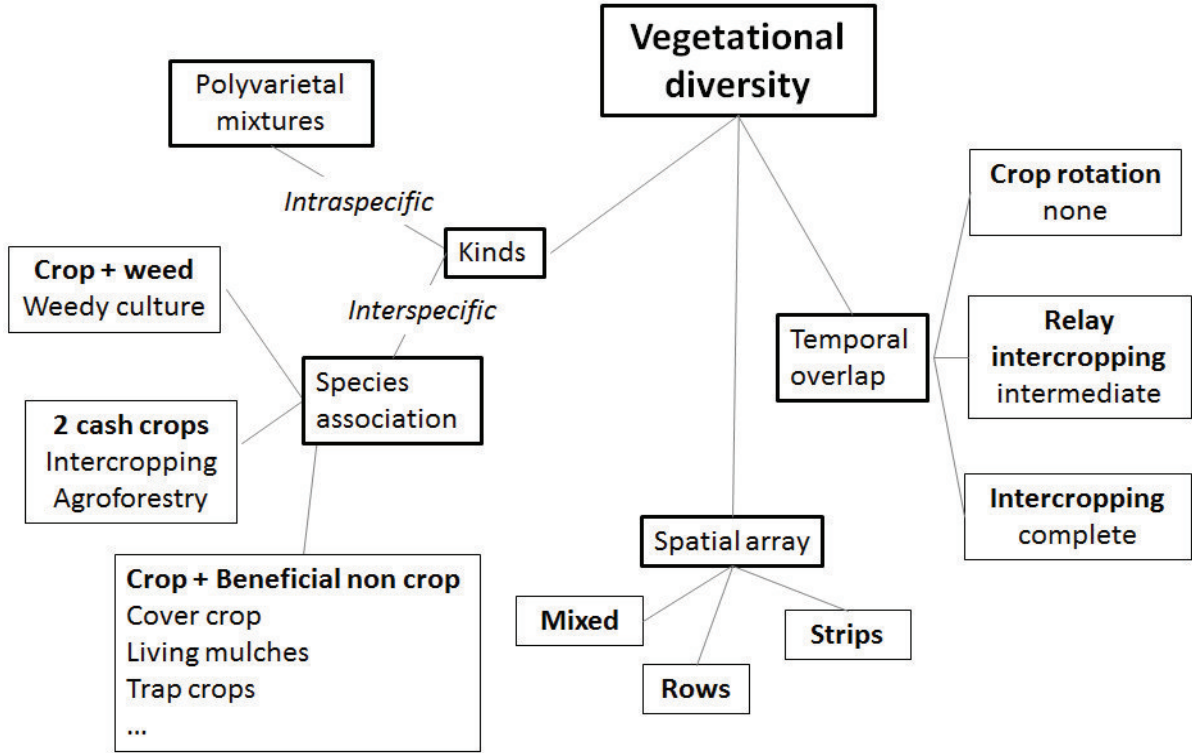


Figure 1.5: Different forms of vegetational diversification within agricultural fields.

### 1.3.1 Intraspecific diversification

#### *Practices*

Intraspecific diversification typically consists in manipulating the number of genotypes in a plant population (Koricheva & Hayes, 2018). In an agroecosystem, it involves the cultivation of several cultivars of a crop species. For example, genetic diversity in wheat mixture varies from 2-5 numbers of components (Borg et al., 2018). As a farming practice, it has been studied from the eighteenth century, first for its overyielding potential and then for disease regulation (Koricheva & Hayes, 2018; Borg et al., 2018). Relatively suitable for mechanized cropping systems such as the cultivation of small grains, cultivar mixtures represent an interesting alternative practice. This may apply especially for low input cropping systems which represent currently several thousands of hectares in Europe (Finckh et al., 2000; Tooker & Frank, 2012; Reiss & Drinkwater, 2018; Borg et al., 2018). Besides, some studies have discussed the interest of polyvarietal mixtures of genetically modified (GM) resistant and non-GM susceptible varieties to slow down the development of insect resistance to transgenic technologies (Onstad et al., 2011; Grettenberger & Tooker, 2015).

#### *Pest regulation*

Little research has been done so far on the effects of intraspecific diversity on arthropod pests and natural enemies (Koricheva & Hayes, 2018) and especially on wheat pest control (Tooker & Frank, 2012; Barot et al., 2017). Studies on the influence of plant genetic diversity on arthropods has mainly targeted herbivores, which are most of the time less abundant in crop cultivar mixtures compared to crop with a single cultivar (Koricheva & Hayes, 2018). In cereal mixtures particularly, herbivores are either reduced or not influenced by the mixture of cultivars compared to monocultures (Table 1.1). This variability may be explained by the fact that cultivar mixture might be effective only on certain pest species of a crop (Pan & Qin, 2014).

**Table 1.1: Summary of articles that report the effect of intraspecific diversification practices in cereal crops on herbivores, natural enemies and/or production.**

References	Country	Mixture components	Conditions of study	Size of the plot	Crop	Effect of the diversification practice on		
						Herbivore	Natural enemies	Production
Chateil et al., 2013	France	>31	Field trials	3600 m <sup>2</sup>	Winter wheat	-	↗ spider diversity ↗ linyphiid abundance	-
Grettenberger & Tooker, 2016	USA	4	Lab experiments	Pots	Spring wheat	= aphid abundance and preference	↗ lady beetles preference and tenure time	= vegetative and reproductive biomass
Grettenberger & Tooker, 2017	USA	3	Lab experiments	Pots	Winter wheat	↘ offspring of aphid	-	↘ vegetative biomass (4%)
Li et al., 2018	China	2 (but different proportion)	Field trials	100 m <sup>2</sup>	Rice	↘ plant hoppers abundance	-	= yield (when resistant cultivar ≥80%)
Ninkovic et al., 2002	Sweden	2	Field trials	1 m <sup>2</sup>	Spring barley	↘ aphid acceptance	-	-
Ninkovic et al., 2011	Sweden	2	Field trials	24.5 m <sup>2</sup>	Spring barley	= aphid abundance	↗ lady beetles abundance	-
Power, 1991	USA	2	Field trials	15 m <sup>2</sup>	Oat	↘ bird cherry oat aphid (1 year out of 3) = English grain aphid	-	↘ seeds per plant
Shoffner & Tooker, 2013	USA	3 and 6	Lab experiments	Pots	Winter wheat	↘ aphid (only six-line mixtures)	-	↗ vegetative biomass (only six-line mixtures)

For example, a gene of resistance to wheat midge has been identified in wheat cultivars (Vera et al., 2013), and including such cultivars in a mixture may improve the resistance to pests, as observed for the diseases (see below). With regard to aphids, no gene of resistance was identified so far in modern hexaploid wheats (Dedryver et al., 2010). The mechanisms underlying the regulation of pests in cultivar mixtures may therefore differ according to the pest species. Additionally, some examples suggested that a minimum level of pest pressure is necessary to profit from the potential of intraspecific diversification to regulate arthropod pest compared to cultivar monoculture (Power, 1991; Vera et al., 2013).

According to Koricheva & Hayes (2018), abundance of natural enemies was unaffected in crop mixtures. But other studies reported enhancement of natural enemies in spring cereals (Ninkovic et al., 2011; Grettenberger & Tooker, 2017) or soybean fields (Pan & Qin, 2014). In a wheat field, species richness of spiders and abundance of Lyniphiidae spiders were increased by cultivar mixtures related to a taller and more ramified vegetation layer (Chateil et al., 2013). No overall effects of genetic diversity was reported so far on level of predation, parasitism or plant damage (Koricheva & Hayes, 2018).

#### *Other ecosystem services*

Increasing intraspecific diversity has been primarily studied to enhance diseases control, because varieties of a specific crop exhibit slightly different resistance genes to disease, unlike plant resistance to aphids. The monoculture of a single host genotype may therefore favor the selection of pathogens that are able to overcome the resistance (Finckh et al., 2000). Consequently, a diversified pool of crop genotypes demonstrates a better resistance to diseases and a more stable yield (Finckh et al., 2000; Zhu et al., 2000; Mundt, 2002). One of the main mechanisms behind this phenomenon is the dilution effect resulting from an increased distance between host plants with the same susceptibility (Finckh et al., 2000; Mundt, 2002). Concerning diseases with several genetic variants, an avirulent pathogen variant may induce resistance in a variant-specific susceptible variety of a crop by stimulating



the plant defenses (Finckh et al., 2000; Mundt, 2002). In a large scale study on rice blast, mixtures of rice varieties had a more diverse pathogen population compared to monoculture, and the yield of the susceptible variety in mixture was increased by 89% (Zhu et al., 2000). As for pest control, it is argued that on the long term, a crop with more diverse genotypes may slow down the adaptation of pathogen to crop resistance (Zhu et al., 2000). Similarly, mixtures of varieties can bring simultaneous resistance to a cocktail of diseases (Finckh et al., 2000). A major concern of studies on diversification practices to control diseases is the spatial scale of the investigation. Interplot interference is very likely to misrepresent the results in such studies because the distance between susceptible monocultures and mixtures is too small and diseases may spread artificially (Mundt, 2002).

Cultivar mixtures containing varieties with different abilities in term of weed competition may reduce weed pressure or increase the tolerance to weed competition (Kaut et al., 2009; Kiær et al., 2009; Tooker & Frank, 2012; Lazzaro et al., 2018). Rather than diversity, the functional traits of individual cultivars, characterized by morphological attributes such as plant height, early vigour, tillering capacity and canopy architecture, are associated with wheat competitive ability against weeds (Andrew et al., 2015; Lazzaro et al., 2018). The potential of cereal mixtures for weed control has been overlooked so far.

Finally, meta-analyses reported that winter wheat mixtures may produce 4% to 6% higher yields compared to its varieties in pure stand (Kiær et al., 2009; Borg et al., 2018). It is argued that cereal cultivar mixtures present yield and grain protein content advantages especially under low input farming (Sarandon & Sarandon, 1995; Kiær et al., 2012). Increase in cereal grain yield might be dependant of both the number and the proportion of components in the mixture (Kiær et al., 2009). If overyielding is not always observed from wheat cultivar mixing, crop performance might be improved overall when considering water use efficiency (Fang et al., 2014) or grain quantity and quality as well as weed suppression

(Lazzaro et al., 2018). And low input farming often targets an overall performance in term of ecosystem services and amenities rather than overyielding *per se* (Barot et al., 2017).

### 1.3.2 Interspecific diversification

#### *Practices*

Interspecific diversification covers a wider range of farming practices (Figure 1.5) and refers to the association of different species of plants within the field, such as two crops (intercropping strictly speaking) or a cash crop and a non-crop beneficial plant also called companion cropping (Willey, 1979; Ben-Issa et al., 2017; Verret et al., 2017). Despite originally the term “intercropping” was used to cash crops (Willey, 1979), it is nowadays generally used to refer to any association of two or more plant species. According to this larger definition, companion crops are not aimed to be commercialized, contrarily to the cash crop (Verret et al., 2017). Such practices of intercropping, are very ancient and still common in developing countries, where small scale farming dominates (Lithourgidis et al., 2011). In Europe, the practice is rather uncommon in mainstream agriculture, except for agroforestry systems, but there is a renewed interest in particular in the context of organic farming (Brooker et al., 2015). Concerning annual cropping systems, intercrops are mainly composed of plant species from different families (Lithourgidis et al., 2011). Wheat particularly can be associated to a wide range of other plant including legumes such as bean, alfalfa or pea; vegetables such as cucumber, chili pepper, oilseed rape or potato; or other cereals such as maize or barley (Aziz et al., 2015; Lopes et al., 2016).



There is a broad range of different companion plants for which the primary objective is to regulate pests (Table 1.2), but their adoption by farmers remains limited because their implementation are constraining or too costly (Tschardt et al., 2016). An alternative lies in the use of cover crops or other "agroecological service crops", primarily implemented for erosion and weed control, or green manure (Canali et al., 2015; Holland et al., 2016). Cover crop is defined as "any living ground cover that is planted into or after a main crop and then commonly killed before the next crop is planted" (Hartwig & Ammon, 2002).

**Table 1.2: Definitions of the different companion plant used for pest control.**

<b>Practice</b>	<b>Definition</b>	<b>Sources</b>
Banker plant	Banker plant systems typically consist of a non-crop plant that is deliberately infested with a non-pest herbivore. The non-pest herbivore serves as an alternative host for a parasitoid or predator of the target crop pest.	Frank (2010)
Barrier plant	An ideal plant barrier should be a non-host for the virus and the vector, but appealing to aphid landing and attractive to their natural enemies and should allow sufficient residence time to allow aphid probing before taking-off occurs.	Hooks & Fereres (2006)
Indicator plant	A species or variety which makes early detection of pests easier inducing a better cost efficiency in crop management.	Parolin et al. (2012)
Insectary plant	A flowering plant which attracts and possibly maintains, with its nectar and pollen resources, a population of natural enemies.	Parolin et al. (2012)
Repellent plants	A repellent plant is an intercropping culture which repels pests and/or pathogens because of the chemicals emitted by these plants.	Parolin et al. (2012)
Trap crop	Plant stands that are grown to attract insects or other organisms like nematodes to protect target crops from pest attack, [...] preventing the pest from reaching the crops or concentrating them in a certain part of the field where they can be economically destroyed.	Hokkanen (1991)

Weeds may also be manipulated in order to manage arthropod pests and sustain natural enemies (i.e. weedy culture), but their potential are greater in perennial compared to annual cropping systems (Andow, 1991; Norris et al., 2000).

## *Pest regulation*

Several reviews covering a large range of cropping systems have tried to give an overview of how increasing interspecific diversity may benefit pest regulation through natural enemies (Risch, 1983; Andow, 1991; Poveda et al., 2008; Letourneau et al., 2011; Dassou & Tixier, 2016; Lichtenberg et al., 2017). Focusing on wheat, Lopes et al. (2016) reported that research on intercropping systems for biological pest control was scarce in Europe with only four papers referring to such experimentations.

Intrafield diversification has been shown to reduce herbivore abundance in some reviews (Risch, 1983; Letourneau et al., 2009). But a more recent meta-analysis reported no effect of intrafield diversification on herbivore abundance or richness (Lichtenberg et al., 2017). A possible explanation of such contrasted results is that meta-analyses tend to mix up pest and crop species, but also spatial arrangement of both targeted crop and companion planting, or scale and country of field experiments. For example, difference in herbivores' degree of specialization should be considered when compiling studies, because generalist and specialist herbivores are not responding in the same way to interspecific diversification (Dassou & Tixier, 2016). Also concerning the spatial arrangement of the intercrops, success in reducing pests in wheat was found more frequently in strip intercropping compared to relay or mixed intercropping, which was also the less common type of association (Lopes et al., 2016).

Overall, natural enemies, both predators and parasitoids, are not influenced by intrafield diversification, neither in term of abundance nor richness (Dassou & Tixier, 2016; Lopes et al., 2016; Lichtenberg et al., 2017). When considering different type of intrafield diversification practices, Letourneau et al. (2011) reported increased abundance of natural enemies by intercropping, but not by other type of practices such as trap crops or other beneficial non-crop plants. Here again, contrasted observations may result from different responses to diversification according to the natural enemy and / or the type of interspecific

diversification. Among generalist natural enemies, abundance of spider increases in response to intrafield diversification especially when interspersed (e.g. strips or rows) (Sunderland & Samu, 2000). Sowing wheat within a living mulch of white clover also increased spider web densities (Gravesen, 2008). Ground arthropods including carabids and staphylinids were generally found in higher density in weedy culture and intercrops, but if some species benefited from diversification, others did not (Kromp, 1999). Among foliage-dwelling predators, ladybirds were found in higher abundance in wheat-mung bean and wheat-oilseed rape intercrops while they were not influenced by wheat-pea intercrops (Wang et al., 2009; Xie et al., 2012; Lopes et al., 2015).

Studies investigating the pest control potential through actual measures of predation or parasitism are rare (Sunderland & Samu, 2000). Parasitism is one of the primarily investigated estimates of the biological control service, because it is easy to observe in parallel to pest monitoring. Letourneau et al. (2011) reported increased parasitism by intercropping, push-pull systems and intrafield flower patches. Higher parasitism of aphid pest was found in wheat-oilseed rape and wheat-alfalfa intercropping compared to monoculture (Ma et al., 2007; Wang et al., 2009). It is important to notice nevertheless, that the mechanisms leading to a reduction of aphids such as barrier effects and host plant dilution, may also negatively impact natural enemies searching for preys or hosts and thus counteract a top down regulation (Wratten et al., 2007). For example, lower parasitism of aphids was found in broccoli crop grown with living mulches (Costello & Altieri, 1995). Besides parasitism, little is known on the effective service of pest control resulting from interspecific diversification. Correlation between natural enemies and pest abundance is generally assumed to describe an enhanced pest regulation, but it is far from being sufficient because the three trophic systems are highly complex (Chisholm et al., 2014). For example, several studies reported an increase in carabid beetle abundance without positive consequences on pest regulation (Kromp, 1999).

### *Other ecosystem services*

Increasing interspecific diversity might help to regulate diseases within fields (Trenbath, 1993; Lithourgidis et al., 2011). As for cultivar mixtures, dilution effect is one of the main underlying mechanisms resulting from an increased distance between host plants with the same susceptibility (Finckh et al., 2000). Boudreau (2013) reviewed studies comparing disease incidence in intercropped systems and found that intercropping two cereal species or a cereal and a legume species decreased various diseases incidence, among which foliar fungi and oomycetes are the main pathogens. Viruses were reduced in intercropped systems in 70% of the cases. Interestingly, many of the viruses are transmitted by arthropod vectors and intercropping might represent efficient barrier to the vector and consequently to virus spread (Hooks & Fereres, 2006). However, efficiency in disease regulation varies according to species combinations and locations (Boudreau, 2013).

Interspecific diversity has also been shown to provide weed control advantages over sole crops, especially in cereal cropping systems (Liebman & Dyck, 1993; Lithourgidis et al., 2011). Complementarity or facilitation processes between associated plants might result in greater use of resources, consequently reducing the availability in nutrients and light required by weeds (Liebman & Staver, 2001). Here again, the efficiency in weed control is depending on the species associated. For example, intercropping cereal and grain legumes reduces significantly weed biomass compared to legume monocultures, but not compared to cereal monocultures (Bedoussac et al., 2015). Cereal-legumes associations are especially promising to control weed, as demonstrated by Verret *et al.* (2017) reporting lower weed biomasses in 82% and 66% of the cases when compared to non-weeded and weeded controls respectively.

Finally, intercropping systems may improve productivity in term of yield per unit area, namely due to a complementary use of resources, facilitation, and / or increased pest regulation (Brooker et al., 2015). Intercropping, especially in the case of two cash crops, usually aims to increase the production of both crops for an optimization of the crop area.

Land equivalent ratio is frequently used as an indicator of agronomic performance of intercropped systems, and is defined as "the relative land area required as sole crops to produce the same yields as intercropping" (Mead & Willey, 1980). But several other indicators might be used as "aggressivity" or "cumulative relative efficiency index" (Bedoussac & Justes, 2011). Higher yields and protein content were found in cereal-grain intercrops over 58 studies (Bedoussac et al., 2015). Yield stability is also enhanced in intercropped systems over three years or more (Raseduzzaman & Jensen, 2017). In intercropping systems where the second plant is a not a cash crop (i.e. companion cropping), the production service is not the main target, even if yield should not be decreased by the companion crop (Verret et al., 2017). The objective is to provide economic or environmental benefits, such as decreasing the risk of crop failure or biotic pressures and improving soil fertility (Lithourgidis et al., 2011). Companion cropping resulted in lower yields in cereal-legumes associations in approximately 50% of the cases, but "win-win" situations dominated when considering trade-offs between yield and weed regulation (Verret et al., 2017). Another meta-analysis reported difference in yields according to additive and substitutive designs, and if the secondary crop was a legume or not and if it was harvested or not (Iverson et al., 2014). They also report trade-offs between yield and pest control in substitutive designs.

#### **1.4 Ecostacking: stacking intra- and inter-specific diversity**

The link between biodiversity and ecosystem functioning has long been accepted and many experimental studies established that diversity is a key determinant of ecosystem processes (Naeem et al., 2002; Tilman, 2015). As reviewed above, both intraspecific and interspecific diversity in cropping systems may benefit the delivery of ecosystem services. However, the majority of these studies have been conducted in separate systems, and the potential interactions between the two levels of diversity have being largely overlooked so far in cropping systems (Hokkanen & Menzler-Hokkanen, 2018; Koricheva & Hayes, 2018). To

enhance ecosystem services in cropping systems, and especially pest control, the ecostacking approach proposes *"combining in a synergistic manner the beneficial services of functional biodiversity from all levels and types"* (Hokkanen, 2017). In other words, it aims at associating several ecosystem service providers in order to optimize the delivery of ecosystem services. Ecosystem service providers may be an organism, an interaction network, or even a habitat (Kremen, 2005; Gurr et al., 2017). In this context, intraspecific and interspecific diversification may each represent an ecosystem service provider. The push-pull system is a successful example of increased pest control due to the combination of two ecological strategies (Khan & Pickett, 2004).

Only few studies have investigated the influence of manipulating simultaneously both intra- and interspecific diversity of host plants on herbivores and their natural enemies, but none concerned annual cropping systems (Cook-Patton et al., 2011; Moreira et al., 2014; Campos-Navarrete et al., 2015; Hahn et al., 2017). Ecostacking may result in additive effects that are the resulting addition of arthropod responses to each single components present in the diversified stand, or in non-additive effects, that are not predicted by such addition but is the result of interactions among the components present in the diversified stand (Johnson et al., 2006). Non additive effects might result in synergy if the effects are positive overall or in antagonism if negative overall. For example, increasing genetic diversity of sub-tropical trees increased herbivory when grown in tree species mixtures but there was no effect from genetic diversity alone (Hahn et al., 2017).

## **1.5 Research questions:**

### 1.5.1 Problem statement

Increasing diversity at the intraspecific (genetic) or interspecific (species) level within cereal cropping systems are promising diversification practices. Such practices have a high potential for implementation by farmers, provided they offer multiple ecosystem services besides controlling pests, and that they are technically not too constraining for the farmer. However, the literature review and the meta-analyses concerning the impact of intrafield diversification on pest control highlight the large variability in the results concerning herbivores, natural enemies, as well as the regulation function itself depending of the practices tested.

It is therefore essential to identify diversification practices that have a good potential to be implemented by the farmers because they deliver multiple ecosystem services, and that may also increase the ecosystem service of pest regulation. Moreover, combining both intra- and inter-specific diversification practices at the field scale could potentially result in an optimization of their potential to control pests. However, this has never been verified in annual cropping systems and under real farming conditions.

### 1.5.2 Selected intrafield diversification practices

In this work, we focus on the common wheat (*Triticum aestivum L.*) because it is among the most widely grown cereal crops worldwide (Tilman et al., 2002) and the most cultivated cereals in the region studied (Auvergne-Rhone-Alpes) in terms of land extension (FranceAgriMer, 2017). We selected two intrafield diversification practices that meet the following criteria: (1) delivering multiple ecosystem services, (2) already being implemented by at least some farmers and (3) having potential for pest control. The two selected practices were wheat cultivar mixtures and wheat clover intercropping. Each practice has a good

potential for pest regulation as shown below, but validation of the theoretical principles by field studies are largely missing.

*Intraspecific diversification: wheat cultivar mixtures*

As described above, mixtures of cereal cultivars, especially wheat, knows a new surge of interest and provide yield advantages as well as enhanced biotic and abiotic resistance (Kiær et al., 2009; Lazzaro et al., 2018; Borg et al., 2018). Wheat cultivar mixtures can be qualified of common practice as it represents almost 50 % of the wheat fields in Europe (Tooker & Frank, 2012). Wheat mixtures may produce 5 % higher yield in condition of competition for light, soil and water resources and up to 30 % higher yields in case of disease pressure compared to monoculture (Tooker & Frank, 2012). The potential for pest control has been investigated in laboratory studies demonstrating positive effect of genetic diversity on aphid regulation through bottom-up and / or top-down processes (Shoffner & Tooker, 2013; Grettenberger & Tooker, 2016, 2017). But none investigated this ecosystem services under real farming conditions, which is among the aims of this work.

*Interspecific diversification: wheat-white clover intercropping*

Increasing the cultivation of legumes for animal feed or for food and other ecosystem services (e.g. supply of nitrogen) is a growing ambition in agriculture (Stagnari et al., 2017). In this relation, intercropping cereals and legumes has several agronomical and environmental advantages (Bedoussac & Justes, 2010a; Lithourgidis et al., 2011; Pelzer et al., 2012; Bedoussac et al., 2015), including the reduction in numbers of cereal and legume aphids (Ndzana et al., 2014; Lopes et al., 2015; Hatt et al., 2018; Xu et al., 2018). There is a great need for experimental trials of new combinations (Brooker et al., 2015). Combining wheat with a legume-based cover crop for pest control has however received little attention so far (Lopes et al., 2016) and data or simulation models are lacking to estimate cover crop effects on services such as pest and beneficial insect activity (Schipanski et al., 2014). The simultaneous intercropping of wheat and clover provides multiple ecosystem services (i.e.



weed control and nitrogen fixation) without impacting grain yield (Vrignon-Brenas et al., 2018) but its potential for pest control remains to be investigated.

### 1.5.3 Objectives of the thesis

The core aims of the thesis are to investigate the potential of each practice (i.e. wheat cultivar mixtures and wheat intercropped with white clover) to control a specific pest, and to bring the ecostacking principle face to face with the reality of the field conditions by combining both practices. These aims were pursued via the following objectives:

#### **Objective 1: Determine the influence of intraspecific and interspecific diversification practices on pest populations of aphids in wheat fields**

According to the resource concentration hypothesis (Root, 1973), specialized herbivores are more likely to find and remain on concentrated host plants. Intraspecific diversification may introduce variation in traits affecting the aerial architecture of wheat cultivar mixtures and may influence the microclimate, thus disfavours the pest population (Barot et al. 2017), while interspecific diversification may impede aphid landing due to increased ground cover by non-host plant biomass (Bottenberg and Irwin 1992; Finch and Collier 2000). For the work carried out in this thesis, we hypothesize therefore that aphid populations will be reduced by each single diversification practice (*Hypothesis 1*) and in a synergistic way by the double diversification scheme consisting of both intraspecific and interspecific diversification (*Hypothesis 2*).

We also want to determine the impact of diversification practices on the agronomic performance of the crop (production service), an essential parameter to consider for farmers to adopt practices. Based on former studies on the same practices (Kiær et al., 2009; Vrignon-Brenas et al., 2018; Borg et al., 2018), we hypothesize that cereal grain yield and nitrogen content will be as good in the diversification treatments as in genetic and species

monoculture, except for wheat-clover intercropping, for which nitrogen content may be reduced (*Hypothesis 3*). These hypotheses are verified under real farming conditions to assess the real extent of the impact of diversification practices on the level of pest infestation and agronomic performance.

**Objective 2: Assess the impact of two different non-host plant in wheat-based intercrops on individual host location abilities and population growth of cereal aphids.**

One mechanism to explain the success of intercrop diversification to control pests is that aphids' host plant location is reduced in the presence of a non-host plant that hides or impedes the physical access to the host plant (Perrin & Phillips, 1978; Finch & Collier, 2000). We hypothesize that aphid host location will be reduced in the presence of a non-host plant (*Hypothesis 4*), and test this under controlled laboratory conditions. We further hypothesize that consequently to failure in host finding, aphid population growth will be reduced in the presence of a non-host plant (*Hypothesis 5*). In wheat-based intercropping systems, efficiency in pest reduction varies with the species used as intercrop (Lopes et al., 2016). We therefore compare here two non-host plants that are each commonly intercropped with wheat and structurally different: white clover and pea (Lopes et al., 2016; Vrignon-Brenas et al., 2018). We hypothesize that the negative effects of diversification on aphid host location and aphid population growth would differ according to the species used as non-host plant (*Hypothesis 6*). In this way we aim at identifying if those parameters that may be responsible for pest control success and may explain the variable results reported in the literature.

**Objective 3: Determine the influence of intraspecific and interspecific diversification practices on natural enemies and their potential of predation on aphids in cereal fields**

Natural enemies are expected to be more varied and abundant in diversified environments (Root, 1973) due to the provision of shelter, nectar, alternative prey/hosts, and pollen, promoting the presence of natural enemies (Gurr et al., 2017). We hypothesize that natural enemies' abundance and diversity on farmer fields are increased by each single diversification practice (*Hypothesis 7*) and in a synergistic way by the double diversification scheme (*Hypothesis 8*). Our field experimental design does not seek to disentangle bottom up vs. top down control. However, we use sentinel preys as an indicator of the potential predation activity that natural enemies may exert on aphid pests. We further hypothesize that in accordance with the abundance of natural enemies, predation and parasitism rates are higher for each single (*Hypothesis 9*) and combined diversification practices (*Hypothesis 10*).

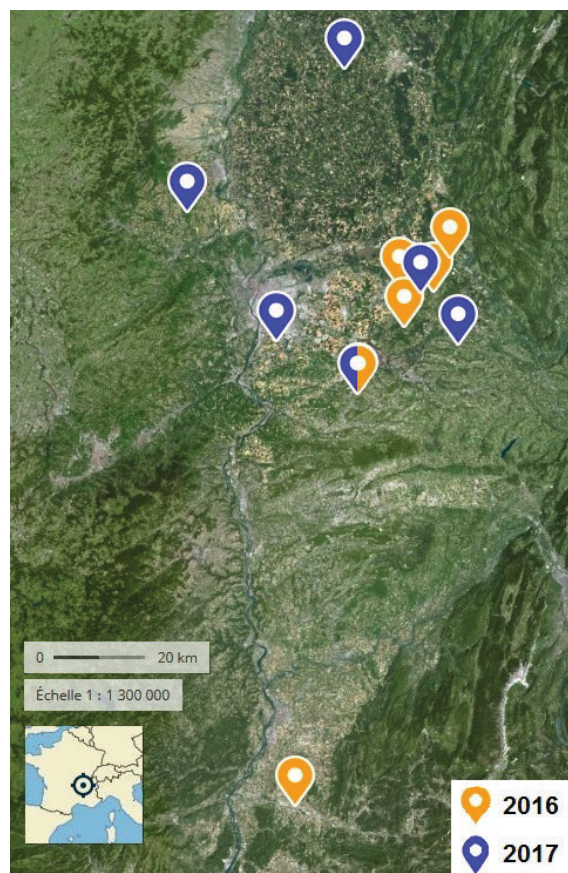
## **Chapter 2 :**

### Methodological approaches

The different methodological approaches used in the field experiments are presented here to provide an overview, because the following chapters are written in form of scientific articles. The methods used will be presented in detail in the respective chapters 3 and 5. The methods used for the laboratory experiments are only presented in the chapter 4.

## **2.1 Experimental sites**

Twelve field experiments mobilizing 10 different farmers were established on organic fields in the southeast of France (Auvergne-Rhône-Alpes region) over two winter wheat cropping seasons (2015–2016 and 2016–2017) (Figure 2.1). The minimum distance between sites of the same growing season was 7 km. The region and the farms where we worked were characterized by low field size (4.5 ha in average and ranking from 1.8 ha to 15 ha), and a landscape composed at 65% by crop lands.



**Figure 2.1: Location of the field experiments. In orange, wheat fields monitored over the 2015-2016 growing season and in blue, wheat fields monitored over the 2016-2017 growing season.**

We chose to work under organic farming conditions initially because according to French organic farming regulations, no pesticides, herbicides, nor fungicides are used on the crops during the experiment. Therefore, we do not have concerns about potential interference between the effect of diversification and the application of pesticides on the arthropods monitored. Moreover, organic farmers are also more prone to implement diversification practices on their cereal fields (David et al., 2012). They are especially interested in diversification practices that may provide multiple ecosystem services, because without chemical inputs, nitrogen deficiency and weed infestation are the two main difficulties of organic cereal farming (David et al., 2012). The association of legume cover crops with cereals has been shown to provide the two necessary ecosystem services: N fertilization and weed control (Hartwig & Ammon, 2002; Scholberg et al., 2010), including the intercropping of wheat with white clover (Vrignon-Brenas et al., 2016b). Wheat cultivar mixtures were also commonly used by the farmers with who we worked. Wheat cultivar mixtures are especially interesting in organic farming for disease control (Finckh et al., 2000).

## **2.2 Arthropods: monitoring of pests and natural enemies**

Aphids are amongst the most essential pest of wheat, and are responsible for considerable wheat crop losses (Dedryver et al., 2010). Three main species are found in Europe: *Sitobion avenae* (F.), *Metopolophium dirhodum* (Wlk.) and *Rhopalosiphum padi* (L.). In winter wheat fields, aphidophagous predators are highly diverse and mainly represented by ground-dwelling arthropods such as ground beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae) and spiders (Araneae) but also vegetation-dwelling arthropods such as cantharids (Coleoptera: Cantharidae), spiders, adults and larvae of ladybirds (Coleoptera: Coccinellidae) as well as larvae of hoverfly (Diptera: Syrphidae) and lacewings (Neuroptera: Chrysopidae) make part of the natural enemy guild in Europe (Sunderland & Crook, 1987; Schmidt et al., 2003; Holland et al., 2008; Choate & Lundgren, 2015). Parasitoid wasps

(Hymenoptera: Braconidae and Ichneumonidae) are also effective predators of aphids but with a high degree of host specialization (Schmidt et al., 2003).

To assess the arthropod community composition within cereal fields, several methods can be used in combination to cover both foliage dwelling and ground dwelling arthropods. And we chose to focus on two of them: visual counting and pitfall trapping.

### 2.2.1 Visual Counting

Probably the easiest and cheapest method to assess the density of arthropods, both pests and natural enemies, visual counting consists of counting individuals *in situ* directly in the field and on crop plants. This method is currently widely used for monitoring aphid populations. In arable cropping systems for example a certain number of host plants are chosen randomly within a plot according to the design of the experiment and then visual observed. Counting of aphids on tillers may varies from 20 tillers for 100m<sup>2</sup> (0.2 tillers per m<sup>2</sup>) (Lopes et al., 2015) to 20 tillers for 0.79m<sup>2</sup> (25 tillers per m<sup>2</sup>) (Schmidt et al., 2003). The number of aphids found is recorded on the whole plant or on specific parts such as the wheat ears (Longley et al., 1997); the first flag leaf of winter wheat (Lang, 2003); the stem of alfalfa (Ximenez-Embun et al., 2014). Visual counting can provide an assessment of the abundance of the population, usually expressed as mean number of aphids per tiller. Several studies distinguished the life stages of the aphids as winged females, wingless females and nymphs of various instars (Chambers et al., 1986; Lopes et al., 2015). Period of monitoring differs among studies according to their objective: every week (Chambers et al., 1986; Lopes et al., 2015), every four days (Xie et al., 2012), every two days every two weeks (Lang, 2003) or at specific wheat growth stages such as flowering and milk ripening (Schmidt et al., 2003, 2004).

This method is also used to record the natural enemies active on the plant during the day but is not well adapted for ground dwelling arthropods such as carabids and staphylinids more active during the night (Jervis, 2005). The abundance of arthropods observed is expressed as the mean number of individuals per host measurements (e.g. plant or tillers).

Visual counting is however labor intensive, dependent on the weather conditions and most efficient for pests and conspicuous groups of natural enemies such as Coccinellidae, Syrphidae and Chrysopidae (Jervis, 2005) or immobile life stage such as larvae or eggs (Chambers et al., 1986; Xie et al., 2012).

Finally, we used visual counting to monitor both aphids and foliage dwelling natural enemies. Only taxa and growth stages with a potential predatory activity were recorded, i.e. adult and larvae of predatory lady beetles (Coccinellidae); larvae of hoverflies (Syrphidae); adult spiders (Araneae), rove and soldier beetles (Staphylinidae and Cantharidae, respectively), and also larvae of lacewings (Neuroptera).

### 2.2.2 Pitfall traps

Pitfall trapping is the most widely used method to sample ground-dwelling arthropods such as carabid and staphylinid beetles, spiders and predatory mites within arable fields (Carmona & Landis, 1999; Lang, 2003; Östman, 2004; Birkhofer et al., 2008). They usually consist in a container of 6 to 10 cm of diameter filled with a preservative liquid (e.g. ethylene or propylene glycol) to both kill and preserve the catch (Jervis, 2005; Thomas, 2008; Winqvist et al., 2011) when the pitfall is not checked every day (Carmona & Landis, 1999). A cover can be associated to the pitfall trap to limit the by-catch (small mammals) or the interference with birds or rain. The traps are almost always open for a period of one week and renewed every two weeks or at specific winter wheat stages as the appearance of spikes and the milk ripening stage (Winqvist et al., 2011). Pitfall traps do not provide data on absolute abundance but on the cumulative activity-density of the sampled arthropods over the sampling period, because the number of individual caught is dependent on their locomotor activity. The activity-density is expressed as the mean number of individuals per traps. But the absence of a species in the catch does not prove its absence in the field (Jervis, 2005).

Finally, we used pitfall trapping to monitor ground-dwelling predatory arthropods i.e., Araneae, Opiliones and Coleoptera.



### **2.3 Assessing the ecosystem service of pest regulation**

Studies concerning the effects of management practices such as habitat manipulation are often limited to the investigation of pest and natural enemies' abundance/activity and provide only a likelihood of biological control and not a proper quantitative measure of the impacts of the targeted pest and its natural enemies (Furlong & Zalucki, 2010; Chisholm et al., 2014; Macfadyen et al., 2015). They rely on the assumption that the presence of more natural enemies equals more biological control. Intraguild predation, hyperparasitism, distraction by other food sources or simply difficulties in prey location and/or access may however lower the impact of natural enemies on herbivore populations (Letourneau et al., 2009). Moreover measures of crop yield is too often absent from biological control studies (Chaplin-Kramer et al., 2011; Chisholm et al., 2014) and may therefore miss out an essential argument in the context of agricultural studies. There is thus a great need to include tools that assess the impacts of the pest and natural enemies on the crop and on each other within biological control studies (Furlong & Zalucki, 2010). The impact of natural enemies on the pest population may be appraised by indirect methods such as sentinel preys, either real or artificial (Lövei & Ferrante, 2017). They are based on the measurement of the removal rate of or the bites left on the sentinel prey to determine the presence and the activity's intensity of natural enemies (Jervis, 2005). Even if such methods come with many bias such as artificially immobility or odourless pests that may modify the natural predation behavior of the predators (Furlong & Zalucki, 2010), they are rather powerful to compare the predation intensity among different habitats (Jervis, 2005; Lövei & Ferrante, 2017) which is among the objectives of our study. In this work, we are especially interested in two sentinel prey methods: predation cards with aphids as sentinel prey and artificial caterpillars.

### 2.3.1 Predation cards

Predation cards consist in artificially clustered sentinel prey tethered on a substrate and exposed to predation in the field for a fixed time. Predation cards are employed most often with eggs (Thomson & Hoffmann, 2010; Balmer et al., 2013; Lövei & Ferrante, 2017), but predation cards with living aphids are also used in different studies (Östman et al., 2001; Östman, 2004; Winqvist et al., 2011; Ximenez-Embun et al., 2014). Predation incidence is measured as the percentage of cards with aphids preyed upon (cards with 1 or more aphids preyed upon/total numbers of cards deployed) and predation intensity as the percentage of aphids preyed upon (aphids preyed upon /total aphids offered) when there are more than a single aphid per card. This method can allow to approximate the pressure of predation exerted by natural enemies at a given time but it cannot reflect the impact of the predators on the pest population dynamic as the sentinel prey used represents only a single life stage (Furlong & Zalucki, 2010).

Finally, we used aphid predation cards, on which was placed one live aphid, to measure the potential predation activity on wheat leaves (Figure 2.2).

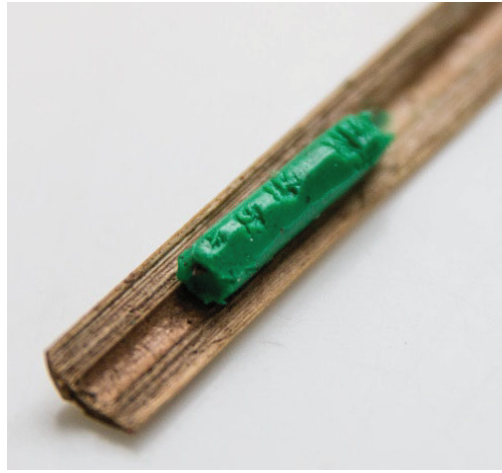


**Figure 2.2: The aphid predation cards used on wheat leaves during this PhD research (for the year 2016). Photos: Agathe Mansion-Vaquié**

### 2.3.2 Artificial caterpillars

Caterpillars can be artificially modelled by using green plasticine and its malleability saves the marks caused by predator's mandibles, teeth, beak, or ovipositor while attempting to predate the sentinel prey (Howe et al., 2009). Artificial caterpillars are in the majority of experiments exposed to predation for 24h and collected to examine the nature of marks on field using a hand-held magnifying glass (20×) or at the laboratory (Howe et al., 2009; Ferrante et al., 2014; Ferrante et al., 2017a; Lövei & Ferrante, 2017). This method allows the identification of up to 14 different types of predators (Low et al., 2014; Lövei & Ferrante, 2017). The choice of the spatial distribution of the caterpillars in the experimental design depends on the crop studied or the targeted pest predators. They can be clustered on the soil surface to provide some repetition within the plot and target the ground dwelling predators or they can be tethered to different substrate such as plant leaf or stem (Lövei & Ferrante, 2017). Predation incidence is measured as the percentage of caterpillars with marks of bites by different predators upon the total numbers of artificial caterpillars deployed. Real sentinel prey is generally assumed to better mimic real prey in the field than fake caterpillars, but some studies temper such assumption. For example the predatory carabid *Pterostichus melanarius* (Illiger) did not show preference for the unwounded alive caterpillars compared to artificial odourless caterpillars made of plasticine (Ferrante et al., 2017b). This means that this method is valuable to estimate the predation pressure in the field.

Finally, we used artificial caterpillars to measure the potential predation activity at the ground level (Figure 2.3).



**Figure 2.3: (A) The artificial caterpillars used on the ground of experimental fields during this PhD research (2015-2017), and (B) The attack marks left by chewing insects on an artificial caterpillar. Photos: (A) Agathe Mansion-Vaquié et (B) Olivier Duchêne.**

## **Chapter 3:**

Wheat genotypic diversity and intercropping to control  
cereal aphids

This chapter corresponds to the article entitled **Wheat genotypic diversity and intercropping to control cereal aphids** (Mansion-Vaquié et al.) submitted for publication in the journal *Agriculture, Ecosystems and Environment* in December 2018.

In this chapter we aim at investigating the influence of intraspecific and interspecific diversification practices on pest populations of aphids in wheat fields. We are particularly interested in the resulting effects from combining both level of diversity within the field, because this has not been reported so far in annual cropping systems. We explore also the influence of each practices and their combination on the agronomic performances of wheat (i.e. grain yield and nitrogen content) because they are determining parameters for the adoption of the practices by farmers.

We remind the hypotheses addressed in this chapter:

- **Hypothesis 1:** aphid populations will be reduced by each single diversification practice: wheat cultivar mixtures (intraspecific diversification) and wheat intercropped with white clover (interspecific diversification);
- **Hypothesis 2:** aphid populations will be reduced in a synergistic way by the combination of both intraspecific and interspecific diversification;
- **Hypothesis 3:** wheat grain yield and nitrogen content will be as good in the diversification treatments as in genetic and species monoculture, except for wheat-clover intercropping, for which nitrogen content may be reduced.

These hypotheses are verified under real farming conditions to assess the real extent of the impact of diversification practices on the level of pest infestation and agronomic performance.

# Wheat genotypic diversity and intercropping to control cereal aphids

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## Abstract

Increasing intrafield plant diversity has been shown to regulate pest populations. Mixing wheat cultivars and intercropping winter wheat and white clover are both promising agroecological practices. On field experiments over two growing seasons, we combined both practices and examined the impact on aphid populations and on wheat production. Results show that combining intra- and interspecific diversity did not outperform each practice individually in reducing aphid populations. Taken separately, intercropping tended to have lower aphid infestation, while it was intermediate in cultivar mixtures. Yearly variation in climatic conditions impacted wheat and clover development, as well as the appearance of aphid peaks. Wheat yields and grain nitrogen content were reduced in intercropping, but not in cultivar mixtures. Our findings suggest that intrafield diversification may regulate wheat aphids to some extent, but combining two diversification practices did not result in an attractive trade-off between pest regulation and wheat production in real farming conditions.

**Keywords:** Biological pest control; Cultivar mixtures; Intraspecific plant diversity; Cover crop; Interspecific plant diversity; Organic agriculture

### **3.1 Introduction**

Agricultural intensification, which includes simplified crop rotations and the removal of non-cropped areas, has led to a reduction in the spatial and temporal heterogeneity of agricultural landscapes at various scales and to increased intrafield uniformity in terms of botanical and structural diversity (Benton et al., 2003; Tschardt et al., 2005). But increasing intrafield plant diversity has been shown to regulate pest populations in various agroecosystems (Hooks & Johnson, 2003; Letourneau et al., 2011; Dassou & Tixier, 2016) by reducing the pest's ability to find and access its host plant (Poveda et al., 2008). Diversification practices at the field level typically consist of mixing different cultivars of a crop species (intraspecific diversity) or mixing different species of plants (interspecific diversity), such as two crops (intercropping) or a crop and a beneficial non-crop plant (cover cropping or living mulches) (Andow, 1991).

Cultivar mixtures have received a new surge of interest in the search for sustainable farming practices, especially for small grains, and is relatively suitable for mechanized system (Finckh et al., 2000; Reiss & Drinkwater, 2018; Borg et al., 2018). In Europe, mixtures of cereal cultivars is not a marginal practice, but rather is used in several thousands of hectares (Tooker & Frank, 2012; Reiss & Drinkwater, 2018). Meta-analyses have reported that winter wheat cultivar mixtures may produce 4.3% to 5.7% higher yields compared to its component cultivars in pure stand (Kiær et al., 2009; Borg et al., 2018) and provide enhanced disease regulation and a reduced impact of abiotic stressors (Finckh et al., 2000; Mundt, 2002; Barot et al., 2017; Reiss & Drinkwater, 2018). Although little research has been done on the effects of intraspecific diversity on wheat pest control (Tooker & Frank, 2012; Barot et al., 2017), some studies have demonstrated that diversity in crop cultivars may reduce pest infestation or damages in cereal fields (Power, 1991; Vera et al., 2013; Li et al., 2018) or in wheat under laboratory conditions (Shoffner & Tooker, 2013; Grettenberger & Tooker, 2017). There is



thus a need to further investigate the potential of wheat cultivar mixtures to control pests at the field scale.

Concerning the practice of interspecific diversification, wheat-based intercropping systems have been shown to reduce pests (Lopes et al. 2016). Intercropping two cash crops (e.g. wheat and oilseed rape, durum or winter wheat and pea) has been shown to be efficient in controlling the abundance of pea and cereal aphids in field experiments (Wang et al., 2009; Ndzana et al., 2014; Lopes et al., 2015). Cover cropping also has promising potential for multifunctional cropping systems (Hartwig & Ammon, 2002; Schipanski et al., 2014). Adding a cover crop may reduce pest outbreaks and increase abundance of natural enemies (Schmidt et al., 2004, 2007; Prasifka et al., 2006). Combining wheat and a legume-based cover crop for pest control, however, has received little attention so far (Lopes et al. 2016). For example, the simultaneous intercropping of wheat and clover provides multiple ecosystem services, such as weed control and nitrogen fixation, without impacting grain yield (Vrignon-Brenas et al., 2018), but its potential for pest control remains to be investigated.

In our study we chose to investigate the two diversification practices: wheat cultivar mixtures and wheat intercropping with white clover intercropping, in real farming conditions. The key to success for farmers' adoption of diversification practices is that they deliver a range of ecosystem services, in addition to pest suppression (Gurr et al., 2017). Moreover, ecostacking (i.e. combining several ecosystem service providers, which might be an organism, an interaction network, or a habitat), may optimize the delivery of ecosystem services (Gurr et al., 2017; Hokkanen, 2017). The combination of two levels of diversification may result in an optimization of the mechanisms underlying biological pest control. For example, intra- and interspecific diversity may influence arthropods through different mechanisms, especially in the case of herbivores (Koricheva & Hayes, 2018). In similar habitat manipulation studies so far, ecostacking intrafield practices and their resulting interactions have been largely overlooked, and the few studies reported concerned tree or dune systems, not annual crops (Koricheva & Hayes, 2018).

Therefore, in this paper we aim to investigate the potential for pest control using the combination of both intraspecific (mixture of wheat cultivars) and interspecific (wheat-white clover intercropping) diversification practices at the field scale in real farming conditions. We hypothesize that aphid populations will be reduced by each single diversification practice, and also by the synergistic effect from both intraspecific and interspecific diversification. Intraspecific diversification may indeed introduce variation in traits affecting the aerial architecture of wheat cultivar mixtures and may influence the microclimate, thus disfavouring the pest population (Barot et al., 2017), while interspecific diversification may impede aphid landing due to increased ground cover by non-host plant biomass (Bottenberg & Irwin, 1992; Finch & Collier, 2000). We therefore consider the canopy features of the crop for potential correlation with the distribution of the aphid population among the diversification levels. Finally we analyze wheat performance (grain yield and grain nitrogen content) to evaluate the potential of multi-level diversification to provide a multifunctional cropping system that is attractive for farmers to adopt at larger scales.

## **3.2 Materials and methods**

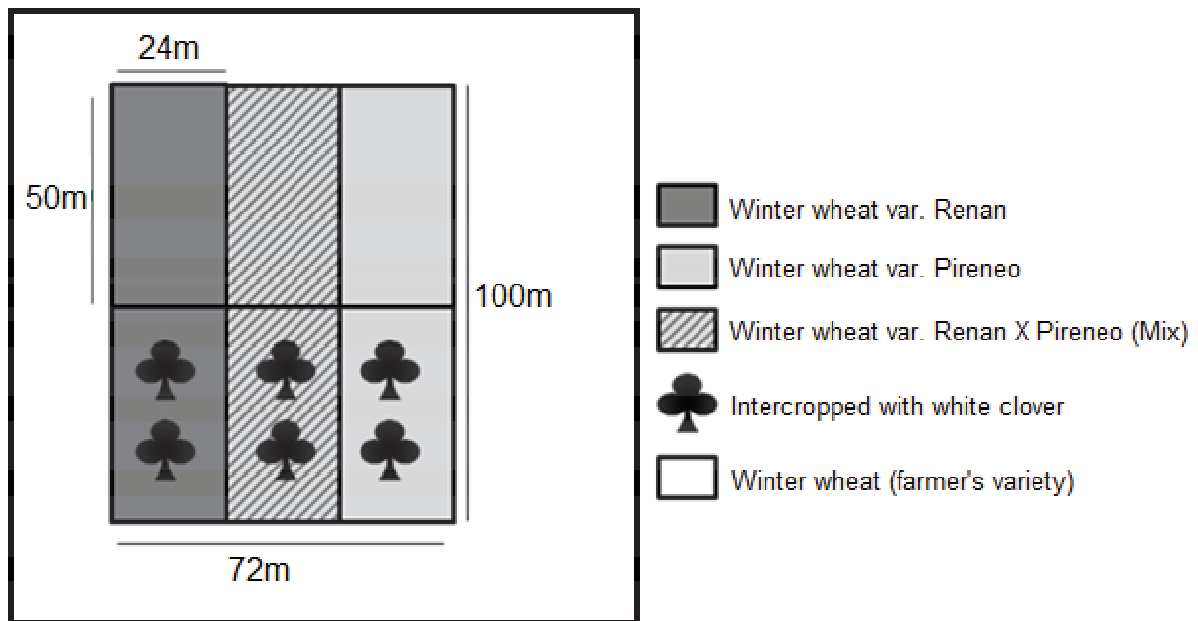
### **3.2.1 Wheat and white clover cultivars**

Two different winter wheat (*Triticum aestivum* L.) cultivars were used in the experiment. The cultivar Renan is considered as the reference cultivar for French organic wheat farming (Dawson et al., 2013). The cultivar Pireneo is another common cultivar for French organic farming; it is on average 16 cm taller and covers less ground than the cultivar Renan (Fontaine et al., 2007). The mixture of wheat cultivars was composed of 50% Pireneo and 50% Renan, and blended in a concrete mixer to ensure homogeneity. With the white clover, we used *Trifolium repens* var. Aberdai in 2016 and *Trifolium repens* var. Rivendel in 2017 (we used different seeds due to provisioning reasons in the different years).

### 3.2.2 Field experiment design

Twelve field experiments were established on organic fields in the southeast of France (Auvergne-Rhône-Alpes region) over two winter wheat cropping seasons (2015–2016 and 2016–2017). Among those fields, only eight could be fully monitored and kept in the analyses: one field in 2016 and three fields in 2017 were excluded because of crop management differences by the farmer and growth problems during crop development. The minimum distance between sites of the same growing season was 7 km. Each field experiment consisted of six treatments: 1) "Renan without clover" - sole stand of wheat monocultivar Renan, 2) "Pireneo without clover" - sole stand of wheat monocultivar Pireneo, 3) "Mix without clover" - sole stand of wheat mixture composed of both cultivars Renan and Pireneo, 4) "Renan with clover" - association of wheat monocultivar Renan with white clover, 5) "Pireneo with clover" - association of wheat monocultivar Pireneo with white clover, and 6) "Mix with clover" - association of the wheat mixture of Renan and Pireneo with white clover. Each treatment was applied on a 1200 m<sup>2</sup> plot (24 m by 50 m) established within an organic wheat field (Figure 3.1).

The 2016 and 2017 fields were sown between October and November in 2015 and 2016, respectively. Winter wheat and white clover were sown simultaneously (with less than a 3-day interval in between) in an additive design at a seed density of 200 kg/ha and 5 kg/ha, respectively (as in Vrignon-Brenas et al. 2016a). In 2017, the white clover had difficulty surviving the winter, and so one field was re-sown with white clover in March 2017. In conformity with French organic farming regulations, no pesticides, herbicides, nor fungicides were used on the crops during the experiment. Following usual farmer practices, weeds were mechanically controlled (one or exceptionally two passages in February-March), except in the treatments with clover.



**Figure 3.1: Schematic representation of the field experimental design.**

### 3.2.3 Sampling of aphids

Aphids were counted directly on wheat tillers every two weeks between March and June in both 2016 and 2017 (for a total of seven observations each year). Each time, 30 wheat tillers were examined in each treatment along a central transect of 30 m, with one tiller every meter. Because it is impossible to distinguish between individual plants of the two wheat cultivars until late in the growing season, it was not possible to determine the distribution of aphids on each of the cultivars individually within the mixture.

### 3.2.4 Vegetation assessments

Wheat height was measured every two weeks between March and June in both 2016 and 2017. The ground cover (percentage of non-bare soil) was visually estimated in six selected quadrats of 0.25 m<sup>2</sup> per treatment every two weeks between March and June in both 2016 and 2017. The ground cover gives an estimation of the porosity of the canopy. The canopy was further characterized by the proportion of wheat and clover cover.

### 3.2.5 Wheat and clover performance

At wheat harvest, six samples per treatment of the aboveground biomass (wheat, clover and weeds) were hand-harvested in 0.25 m<sup>2</sup> quadrats (0.5 m x 0.5 m) and sorted. The collected samples of plants were oven-dried for 48 hours at 60°C and then weighed. To determine the grain yield of the wheat, the straw and the grains were separated and the grain moisture was adjusted to 15% (Vrignon-Brenas et al., 2018). The wheat grains for each treatment and field were milled separately, and then sieved through a 0.1 mm mesh grid. Homogeneous sub-samples of 5 mg were analysed for total nitrogen (N) content using a dry combustion (Dumas method) automatic analyzer LECO CHN-2000 (Leco Corporation, St. Joseph, USA).

### 3.2.6 Statistical analysis

The sum of aphids, i.e. the cumulated number of aphids observed during the whole sampling season per treatment and per field, was used as a global indicator of aphid pressure over the two sampling seasons. The aphid peak is defined as the date of sampling with the highest number of aphids per field and may vary among fields. In 2016, aphid peaks occurred on wheat leaves early in spring (between April and May), during the stem elongation wheat growth stage. In 2017, aphid peaks occurred predominantly on wheat heads late in the spring (between end of May and beginning of June), during different wheat growth stages (from heading to fruit development). The percentage of infestation was defined as the percentage of infested tillers with at least one aphid per treatment of each field at the aphid peak.

All statistical analyses were conducted using R, version 3.4.3 (R Development Core Team, 2017). The measured variables "sums of aphids" and "number of aphids per tiller" were analyzed with a Poisson Generalized Linear Mixed Model (GLMM) with a log link function using the *glmer* function from the *lme4* package (Bates et al., 2015). The variable "percentage of infestation" was analyzed with a binomial GLMM with a logit link function. "Wheat

height", "ground cover", "wheat and non-wheat (combination of clover and weed) biomass", "yield" and "total N content of the grain" were modelled with LMMs using the *lmer* function.

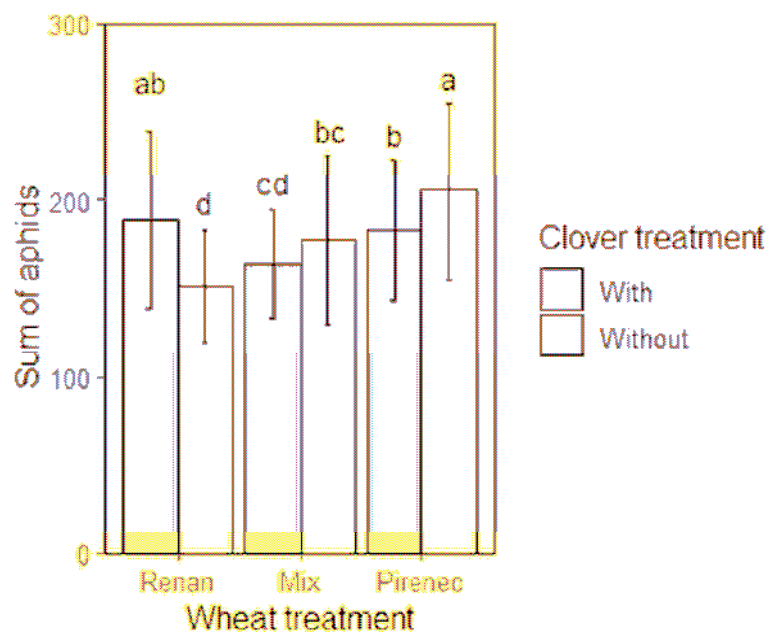
For each of these measured variables, with the exception of "sum of aphids" (see below), six models were fitted with the following fixed covariates: *Wheat* treatment (categorical with three levels: Renan, Pireneo and Mix; the default level was set as Renan, because it is the reference wheat cultivar in French organic agriculture), *Clover* treatment (categorical with two levels: with and without) and *Year* (categorical with two levels: 2016 and 2017). *Field* was used as random factor to consider the dependency among observations of the same field. Model 1 considered all the interactions among fixed effects; Model 2 considered the additive effect of all three fixed-effect variables; Model 3 considered the interaction among diversification treatments with an additive effect of the year; Model 4 considered the interaction among *Clover* treatment and *Year*; Model 5 considered the interaction among *Wheat* treatment and *Year*; Model 6 was the null model. The best model for each measured variable was selected as that with the lowest Akaike information criterion with a second order correction (AICc) adapted for small samples (Burnham & Anderson, 2002).

Because of the small number of repetition (n=8 fields), for the variable "sum of aphids" the Model 3 without *Year* covariate was assigned. The significance of fixed effects from the selected model and their interaction was determined with an F-test with a Kenward-Roger correction for LMMs or likelihood ratio test (LRT) for GLMMs as implemented in the mixed function in the *afex* package (Singmann et al., 2018). Pairwise comparisons were done using Tukey-adjusted *Estimated Marginal Means* (EMMs; a.k.a. least-squares means) with the *emmeans* package (Lenth, 2018).

### 3.3 Results

#### 3.3.1 Aphid populations

Over both sampling seasons, we observed a significant effect of the interaction among both diversification levels (cultivar mixture, intercropping) on the sum of aphids (LRT on GLMM:  $X_{(2,5)}=47.58$ ,  $p\text{-value} < 0.01$ ). Without the clover intercrop, Renan had the lowest sum of aphids and Pireneo the highest, and Mix was the intermediate (Figure 3.2). When wheat was intercropped, Mix was the least infested treatment. The combination of Mix with clover (the most diversified treatment) hosted similar overall numbers of aphids to Renan or Mix without clover.



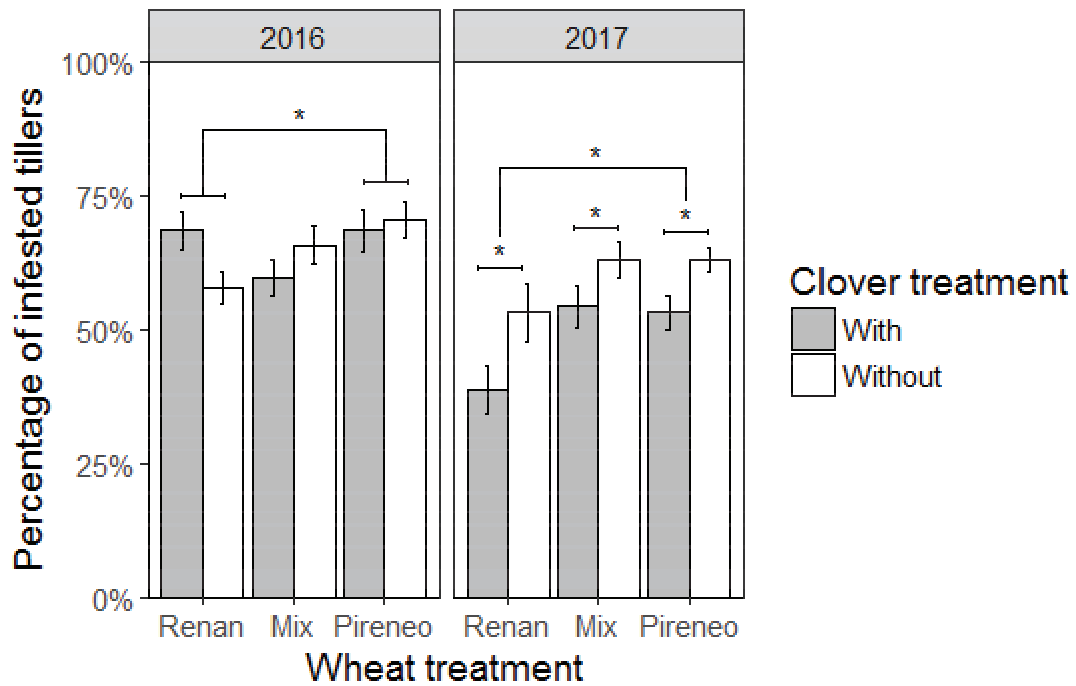
**Figure 3.2:** Sum of aphids (mean  $\pm$  S.E.) over the two sampling seasons, i.e. cumulated number of aphids over 7 sampling dates for each 5 fields in 2016 and 3 fields in 2017 according to wheat treatment and clover treatments. Different letters indicate significant differences according to Tukey-adjusted pairwise EMMS comparisons ( $P\text{-value} < 0.05$ ).

At the aphid peak, the percentage of infestation differed among wheat treatments for both years (Figure 3.3, Table 3.1). Stands of Renan with and without clover were significantly less infested (57 % on average) compared to Pireneo with and without clover (65 % on average) for both years. In 2017 only, wheat (Renan, Pireneo and Mix) intercropped with clover was significantly less infested (by 11 %) compared to wheat without clover.

1 Table 3.1: Results of the (general) linear mixed models (G)LMMs selection relating wheat treatments (W), clover treatments (C) and year (Y) to response variables. Significance of their fixed  
 2 effects and their interaction was determined with F-test with a Kenward-Roger correction for LMMs or likelihood ratio test for GLMMs. Best model for each response variable was selected  
 3 with the lowest AICc value and are underlined. Only the best two models are presented for each response variable.  $\Delta$ AICc represents the difference in AICc with the second closest model.  
 4  $\Delta$ AICc-Null represents the difference in AICc with the null model. Model 1= W\*C\*Y; Model 2= W+C+Y; Model 3= W+C+Y+ W:C; Model 4= W+C+Y+ W:Y; Model 5= W+C+Y+ C:Y;  
 5 Model 6 is the null model. For each model, the variable Field was included as a random effect.

	Response variable	Model	Wheat (W)	Clover (C)	Year (Y)	W:C	W:Y	C:Y	W:C:Y	df	AICc	$\Delta$ AICc - Null	$\Delta$ AICc
Aphid	Percentage infestation	<u>Model 5</u>	$\chi_{(2,5)}= 8.27$ <b>p = 0.02</b>	$\chi_{(1,6)}= 3.90$ <b>p = 0.05</b>	$\chi_{(1,6)}= 0.87$ p = 0.35	-	-	$\chi_{(1,6)}= 5.47$ <b>p = 0.02</b>	-	7	1794.0	6.7	2.0
		Model 1	$\chi_{(2,11)}= 9.19$ <b>p = 0.01</b>	$\chi_{(1,12)}= 3.99$ <b>p = 0.05</b>	$\chi_{(1,12)}= 0.87$ p = 0.35	$\chi_{(2,11)}= 0.93$ p = 0.63	$\chi_{(2,11)}= 4.49$ p = 0.11	$\chi_{(1,12)}= 5.45$ <b>p = 0.02</b>	$\chi_{(1,11)}= 3.55$ p = 0.17	13	1796.0	4.7	
	N aphid per tiller	<u>Model 1</u>	$\chi_{(2,11)}= 49.07$ <b>p &lt; 0.001</b>	$\chi_{(1,12)}= 36.06$ <b>p &lt; 0.001</b>	$\chi_{(1,12)}= 0.06$ p = 0.81	$\chi_{(2,11)}= 24.44$ <b>p &lt; 0.001</b>	$\chi_{(2,11)}= 63.77$ <b>p &lt; 0.001</b>	$\chi_{(1,12)}= 25.20$ <b>p &lt; 0.001</b>	$\chi_{(2,11)}= 65.92$ <b>p &lt; 0.001</b>	13	9390.7	248.0	109.6
		Model 4	$\chi_{(2,6)}= 51.47$ <b>p &lt; 0.001</b>	$\chi_{(1,7)}= 26.81$ <b>p &lt; 0.001</b>	$\chi_{(1,7)}= 0.07$ p = 0.80	$\chi_{(2,6)}= 52.52$ <b>p &lt; 0.001</b>	-	-	-	8	9500.3	138.4	
Vegetation characteristics at aphid peaks	Ground cover	<u>Model 1</u>	$F_{(2,252)}= 1.58$ p = 0.21	$F_{(1,252)}= 28.26$ <b>p &lt; 0.001</b>	$F_{(1,6)}= 1.24$ p = 0.31	$F_{(2,252)}= 0.95$ p = 0.39	$F_{(2,252)}= 0.52$ p = 0.60	$F_{(1,252)}= 5.72$ <b>p = 0.02</b>	$F_{(2,252)}= 4.57$ <b>p = 0.01</b>	14	2113.3	77.2	26.3
		Model 5	$F_{(2,258)}= 1.60$ p = 0.20	$F_{(1,258)}= 27.72$ <b>p &lt; 0.001</b>	$F_{(1,6)}= 1.24$ p = 0.31	-	-	$F_{(1,258)}= 5.61$ <b>p = 0.02</b>	-	8	2139.6	50.9	
	Wheat height	<u>Model 1</u>	$F_{(2,199)}= 32.27$ <b>p &lt; 0.001</b>	$F_{(1,199)}= 1.24$ p = 0.27	$F_{(1,5)}= 17.51$ <b>p &lt; 0.01</b>	$F_{(2,199)}= 0.41$ p = 0.67	$F_{(2,199)}= 56.15$ <b>p &lt; 0.001</b>	$F_{(1,199)}= 0.07$ p = 0.78	$F_{(2,199)}= 0.06$ p = 0.94	14	1380.8	149.5	7.1
		Model 4	$F_{(2,204)}= 33.11$ <b>p &lt; 0.001</b>	$F_{(1,204)}= 1.21$ p = 0.27	$F_{(1,5)}= 17.51$ <b>p &lt; 0.01</b>	-	$F_{(2,204)}= 57.19$ <b>p &lt; 0.001</b>	-	-	9	1387.9	142.4	
Wheat performances at wheat harvest	Yield	<u>Model 4</u>	$F_{(2,275)}= 0.41$ p = 0.67	$F_{(1,275)}= 21.03$ <b>p &lt; 0.001</b>	$F_{(1,6)}= 2.62$ p = 0.16	-	$F_{(2,275)}= 5.06$ <b>p &lt; 0.01</b>	-	-	9	632.1	12.1	2.6
		Model 2	$F_{(2,277)}= 1.40$ p = 0.25	$F_{(1,277)}= 20.44$ <b>p &lt; 0.001</b>	$F_{(1,6)}= 2.62$ p = 0.16	-	-	-	-	7	634.7	9.5	
	N content	<u>Model 3</u>	$F_{(2,35)}= 1.36$ p = 0.27	$F_{(1,35)}= 15.69$ <b>p &lt; 0.001</b>	$F_{(1,6)}= 0.50$ p = 0.51	$F_{(2,35)}= 2.06$ p = 0.14	-	-	-	9	181.5	8.2	0.6
		Model 2	$F_{(2,37)}= 1.29$ p = 0.29	$F_{(1,37)}= 14.84$ <b>p &lt; 0.001</b>	$F_{(1,6)}= 0.50$ p = 0.51	-	-	-	-	7	182.1	7.6	
	Wheat biomass	<u>Model 3</u>	$F_{(2,275)}= 2.07$ p = 0.13	$F_{(1,275)}= 21.11$ <b>p &lt; 0.001</b>	$F_{(1,6)}= 0.71$ p = 0.43	$F_{(2,275)}= 2.67$ p = 0.07	-	-	-	9	1054.7	16.4	0.7
		Model 2	$F_{(2,277)}= 2.06$ p = 0.13	$F_{(1,277)}= 20.9$ <b>p &lt; 0.001</b>	$F_{(1,6)}= 0.71$ p = 0.43	-	-	-	-	7	1055.4	15.7	
Weed biomass	<u>Model 6</u>	-	-	-	-	-	-	-	3	639.7	0	8.7	
	Model 2	$F_{(2,277)}= 3.85$ <b>p = 0.02</b>	$F_{(1,277)}= 0.10$ p = 0.75	$F_{(1,6)}= 0.07$ p = 0.80	-	-	-	-	7	648.4	8.7		





**Figure 3.3: Percentage of colonized tillers infested by aphids at their peak of density in 2016 (n = 150) and in 2017 (n = 90) according to wheat and clover treatments. In 2016 peak of density occurred before wheat flowering, while in 2017 it occurred after wheat flowering.**

The number of aphids per tiller differed among treatments in the years according to a significant three-way interaction (Figure 3.4, Table 3.1). Without clover intercrop and for both years, the abundance of aphids was lowest on Renan, highest on Pireneo and intermediate for Mix. Intercropping wheat with clover significantly decreased the number of aphids per tiller when compared to wheat without clover only for Mix in 2016, while it decreased the size of aphid colonies only for the monocultivars Renan and Pireneo in 2017. The combination of Mix with clover (the most diversified treatment) hosted similar overall numbers of aphids to Renan without clover in 2016, while it was not different from any of the sole stands of wheat in 2017.

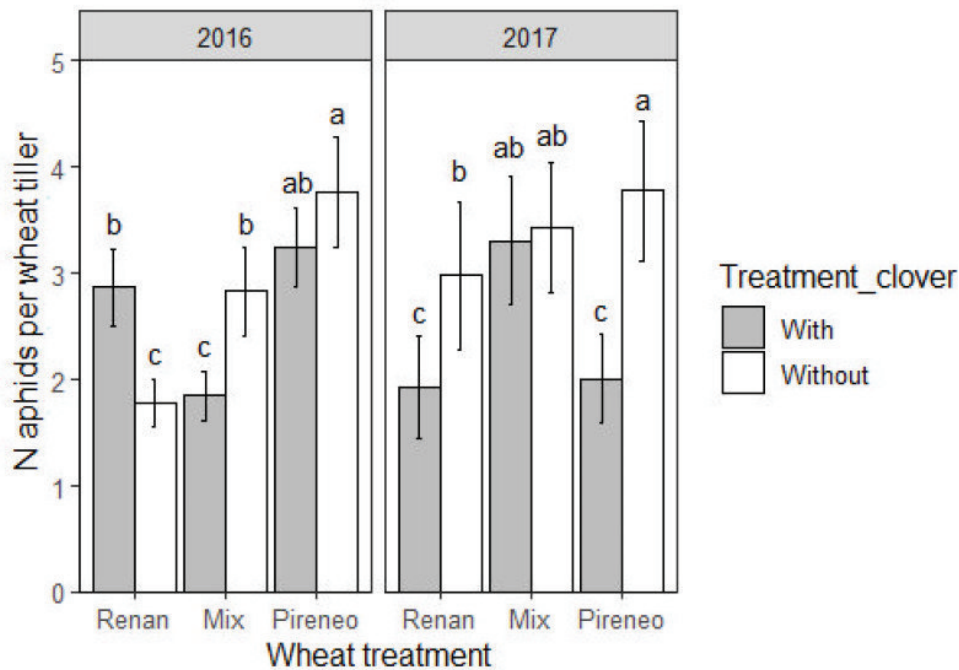


Figure 3.4: Number of aphids per wheat tiller (mean  $\pm$  S.E.) at their peaks in 2016 (n = 150) and in 2017 (n = 90), according to wheat and clover treatments. Different letters indicate significant differences according to Tukey-adjusted pairwise EMMS comparisons per year (P-value < 0.05). In 2016 peak of density occurred before wheat flowering, while in 2017 it occurred after wheat flowering.

### 3.3.2 Change in vegetation cover

The percentage of ground cover was higher in treatments with intercropped clover than without it over the entire season in 2016. The difference in the percentage of ground cover between treatments with and without clover ranged from 12 to 19 % (Figure 3.5). At the aphid peaks in 2016, the ground cover was significantly higher (+15% on average) in the treatments with clover (Table 3.1), and the cultivar Pireneo (with and without clover) had significantly lower ground cover (66%) compared to Renan (73%) and to Mix (72%) (EMMs tests: P-value < 0.05). In 2017, the difference in ground cover was less distinct, and at the aphid peaks the ground cover was similar across all treatments.

Differences in wheat height among treatments were pronounced from the end of May (i.e. the wheat flowering stage) for both years (Figure 3.6). Before this period, differences in the mean wheat height among treatments were less than 5 cm. As a consequence, at aphid peaks in 2016, we observed no difference among treatments. At aphid peaks in 2017, Pireneo was

significantly 5 cm higher than Mix and 17 cm higher than Renan (Table 3.1; EMMs tests: P-value < 0.05). In intercropped treatments, the mean height of white clover at aphid peaks was 9 cm and 12 cm in 2016 and 2017, respectively.

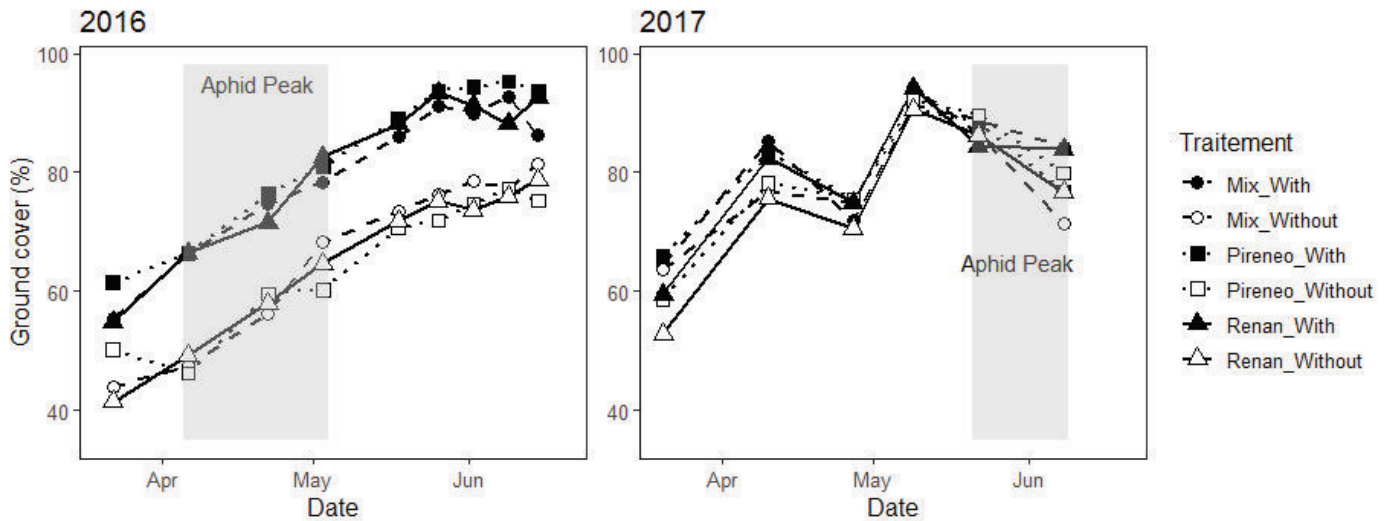


Figure 3.5: Mean ground cover (%) over time for 2016 (n = 27) and 2017 (n = 18) in wheat fields in southeastern France intercropped with clover or not and according to the different wheat treatments. The grey bar represents the period over which the peak of aphids occurred for each field.

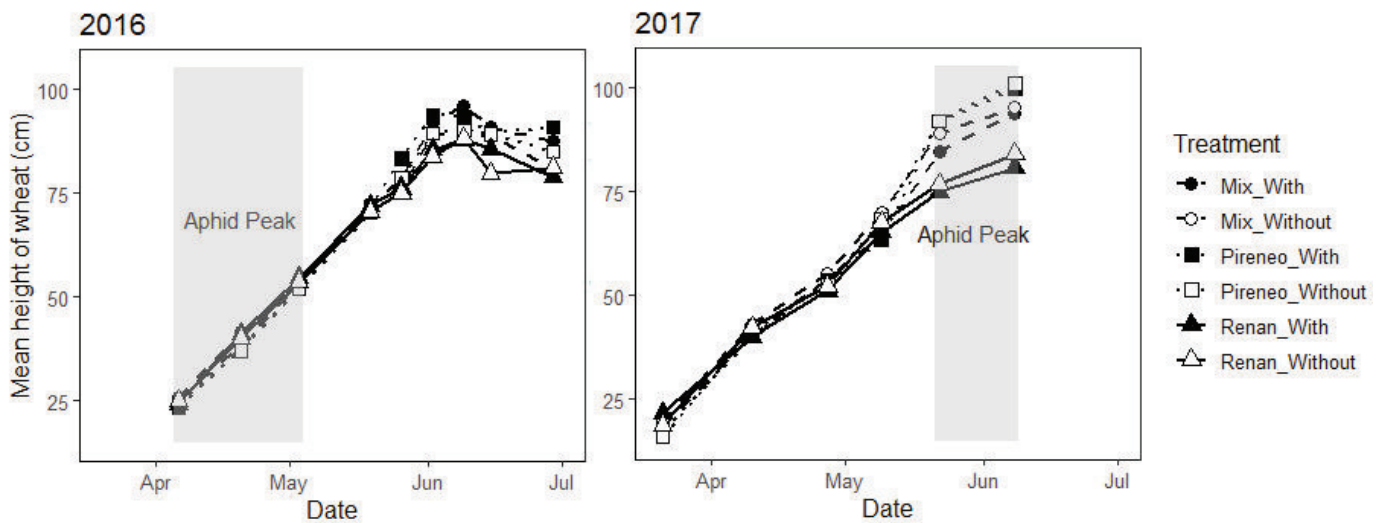


Figure 3.6: Mean height of wheat tiller (cm) over time for 2016 (n = 20) and 2017 (n = 17) in wheat fields in southeastern France intercropped with clover or not and according to the different wheat treatments. The grey bar represents the period over which the peak of aphids occurred for each field.

### 3.3.3 Performance of wheat

We observed a significant interaction between wheat cultivars and years concerning wheat yields. In 2016, Renan (with and without clover) yielded significantly more than Mix and Pireneo (specifically +0.31 t/ha and +0.35 t/ha on average representing an average gain of 12% and 13%, respectively) (Table 3.1, Table 3.2). There was no difference of yields among cultivars in 2017. Yields were significantly lower in wheat stands intercropped with clover compared to wheat monocultures for both years (-0.35 t/ha on average representing an average loss of 10%).

The total N content in the wheat grains at harvest was not significantly different between either years or cultivars (Table 3.1). Intercropping wheat with white clover slightly decreased the total N content in wheat grains (representing an average loss of 7%) (Table 3.2). Wheat aboveground biomass was significantly reduced (-0.74 t/ha on average) for both years when intercropped with clover (Table 3.1, Table 3.2). But biomass of clover at wheat harvest differed strongly between 2016 and 2017, with 1.14 t/ha and 0.10 t/ha on average, respectively. Weed biomass at wheat harvest was not significantly different among treatments or years (Table 3.1, Table 3.2).

**Table 3.2: Wheat and non-wheat biomasses, grain yield and grain total N content (mean  $\pm$  S.E.) collected at wheat harvest in 2016 (n=30) and in 2017 (n=18) in fields in southeastern France, according to wheat treatment and clover treatment. Different letters indicate significant differences per year according to Tukey-adjusted pairwise EMMS comparisons (P< 0.05).**

	Year	Treatments					
		Without clover			With clover		
		Renan	Mix	Pireneo	Renan	Mix	Pireneo
Wheat yield (t/h)	2016	2.91 $\pm$ 0.30 a	2.37 $\pm$ 0.29 b	2.45 $\pm$ 0.21 b	2.40 $\pm$ 0.34 c	2.32 $\pm$ 0.25 d	2.16 $\pm$ 0.27 d
	2017	4.43 $\pm$ 0.47 a	4.55 $\pm$ 0.39 a	4.78 $\pm$ 0.38 a	4.03 $\pm$ 0.41 b	4.20 $\pm$ 0.33 b	4.15 $\pm$ 0.32 b
Total N content (g/kg)*	2016	18.22 $\pm$ 0.64 a	19.72 $\pm$ 0.58 a	19.00 $\pm$ 1.15 a	17.60 $\pm$ 1.33 b	17.32 $\pm$ 1.45 b	17.40 $\pm$ 1.28 b
	2017	16.73 $\pm$ 0.17 a	18.00 $\pm$ 1.05 a	18.33 $\pm$ 1.07 a	16.72 $\pm$ 0.43 b	16.73 $\pm$ 0.69 b	16.89 $\pm$ 0.53 b
Wheat biomass (t DM/h)	2016	7.10 $\pm$ 0.70 a	6.59 $\pm$ 0.72 a	7.26 $\pm$ 0.65 a	6.22 $\pm$ 0.78 b	6.47 $\pm$ 0.70 b	6.27 $\pm$ 0.78 b
	2017	9.03 $\pm$ 1.02 a	9.48 $\pm$ 0.84 a	10.30 $\pm$ 0.86 a	8.37 $\pm$ 0.90 b	8.99 $\pm$ 0.79 b	8.85 $\pm$ 0.75 b
Weed biomass (t DM/h)	2016	1.01 $\pm$ 0.17 a	1.28 $\pm$ 0.22 a	0.92 $\pm$ 0.16 a	1.03 $\pm$ 0.21 a	1.11 $\pm$ 0.25 a	0.94 $\pm$ 0.20 a
	2017	0.70 $\pm$ 0.13 a	1.29 $\pm$ 0.41 a	0.62 $\pm$ 0.15 a	0.94 $\pm$ 0.13 a	0.77 $\pm$ 0.12 a	0.91 $\pm$ 0.15 a

\* Concerning the N content in wheat grains n=5 in 2016 and n=3 in 2017

### **3.4 Discussion**

#### **3.4.1 Intraspecific diversification and aphid populations**

We observed a slight difference in wheat susceptibility to aphids between the two wheat cultivars, with Renan being less infested than Pireneo and the mixture of both cultivars being mostly in between the two when cultivated without a clover intercrop. Contrary to our hypotheses, mixing the two wheat cultivars did not reduce aphid populations in our study. The effect of intraspecific diversification on disease and pest regulation is known to be influenced by the level of biotic pressure (Power, 1991; Huang et al., 2012). The low level of aphid pressure in our study may therefore be among the plausible explanations for the absence of an effect of mixing the wheat cultivars. Indeed, Larsson (2005) defined aphid years as years with aphid populations reaching more than 15 individuals per tiller at aphid peak, occurring cyclically every 3–4 years (Brabec et al., 2014). In our study, peaks of aphids were far from

this threshold, suggesting that the time window of our study may have not covered this phenomenon. Additionally, yearly variation in the potential of pest regulation by mixing cereal cultivars was also found with other experiments. For example on oats, the population of bird cherry-oat aphids (*Rhopalosiphum padi* L.) was decreased by a two-line mixture, but only in one out of three years, and the population of grain aphids (*Sitobion avenae* F.) were not impacted (Power, 1991). The reproduction of such a study on a period longer than two crop-growing seasons should therefore be considered in future research.

Environmental stressors may also be a plausible explanation for the variation observed in our experiments in the potential of the wheat cultivars mixture to regulate aphids. For example, in laboratory experiments, drought has been shown to attenuate the impact of intraspecific diversity on the offspring production by the aphid *Rhopalosiphum padi* on winter wheat (Grettenberger & Tooker, 2016). And in our study, the winter of 2017 showed below-normal precipitation, which may have impacted both plant development and aphid infestation during that year.

Finally, the number of lines in the mixtures may be of importance to trigger effects on aphids. We used a two-line mixture in our study and such simple mixture may have a lower pest regulation potential, as demonstrated in barley with more lines (Ninkovic et al., 2011). In a laboratory study, Shoffner and Tooker (2013) observed that three-line wheat mixtures had similar level of aphid infestation as pure cultivars, but they significantly slowed aphid population growth in the first two weeks of the experiment. Six-line mixtures however, were found with significantly fewer aphids (Shoffner & Tooker, 2013). Rules to design mixtures providing multiple functions are lacking, and identifying appropriate cultivar traits to maximize the functional diversity of wheat mixtures has been proposed to be more important than genetic diversity *per se* (Barot et al., 2017; Borg et al., 2018).

### 3.4.2 Interspecific diversification and aphid populations

We expected a reduction of aphid populations by intercropping wheat and white clover, but our results differed between years. We observed a significant reduction in the percentage of infested tillers and the abundance of aphids on wheat intercropped with white clover only at aphid peaks in 2017 and only in the wheat sole stand treatments. The mechanisms underlying this observation are not straightforward. The clover was indeed underdeveloped at wheat harvest in 2017: the average biomass was 0.10 t DM/ha, which is low compared to 2016 biomass (1.14 t DM /ha) and compared to the average biomass recorded in another study in the region over two years (2.17 t DM/ha on average without fertilization) (Vrignon-Brenas et al., 2018). And there were no significant differences in 2017 between intercropped wheat versus monoculture in terms of ground cover at aphid peaks and of weed biomasses at wheat harvest. Thus, the lower numbers of aphids observed in intercropped plots cannot be simply explained as the result of a higher density or diversity of the aboveground cover.

An alternative explanation that can be considered is a difference in host plant quality. Indeed, nitrogen content in crops is an important parameter to determine host plant quality for aphids (Hanisch, 1980). The nitrogen content in grains is the result of nitrogen being taken up during the flowering growth stage of the wheat until harvest, and its decrease is therefore an indicator of an interspecific competition affecting the nitrogen plant content over this period (Thorsted et al., 2006b). In our study, we observed such a reduction in grain nitrogen content for both years. As aphid peaks occurred after flowering in 2017, aphids on intercropped wheat may have faced a reduced host plant quality that impaired their population growth.

Another factor to be considered is the type of intercropping used in our study that may influence the response of aphids to the increase in interspecific diversity. Indeed, Lopes et al. (2016) reported variabilities in the responses of pests related to the type of intercropping (i.e. strip vs. relay or mixed intercropping) with a reduction of pests in mixed cropping in only half of the cases. For instance, a higher regulation of pests by strip intercropping compared to mix

intercropping was reported in two studies on wheat and pea associations (Ndzana et al., 2014; Lopes et al., 2015). Successful examples of intercropping wheat with strips of alfalfa indicated a reduced infestation of wheat by different aphid species such as *Schizaphis graminum* and *Sitobion avenae* (Ma et al., 2007; Saeed et al., 2013). This suggests that success in pest regulation by wheat intercropping depends also on both the type of intercropping and the pest species targeted (Hooks & Johnson, 2006).

#### 3.4.3 Combining intra- and interspecific diversification practices and aphid populations

Combining wheat cultivar mixture and wheat-white clover intercropping on the same cultivated area did not outperform each practice individually in reducing aphid populations. The most diversified treatment (i.e. wheat cultivar mixture intercropped with clover) was indeed not the least infested one in the study. We did observe, however, interactive effects between intra- and interspecific diversity, although they depended on growing conditions in different years. For instance, the treatment combining both practices of diversification hosted lower aphid populations compared to treatments with no intraspecific diversity in 2016, while it was the opposite in 2017. Such observations were also reported in another study on trees, which found increased herbivory with genetic diversity in tree species mixtures but no effect from genetic diversity alone, and reported variations between years (Hahn et al., 2017).

#### 3.4.4 Heterogeneity in the vegetation layer architecture and aphid population

Although it has been proposed that including variation in traits affecting the aerial architecture influences the microclimate and consequently the pest population (Barot et al., 2017), we did not observe a clear influence from the heterogeneity in vegetation height on aphids. The difference in wheat heights was, as expected, clearly established between the taller cultivar *Pireneo* and the shorter cultivar *Renan*, with the cultivar mixture being intermediate between the two. In our study, the aphid peak occurred on the wheat heads when the canopy height was the most heterogeneous, however this apparently did not impact aphid



populations, because the mixture was among the most infested treatment. Furthermore, ground cover has been shown to reduce aphid landing rates (Bottenberg & Irwin, 1992) by the modification of the visual signal reflected by the cropping system (Wratten et al., 2007). But in our study, increased ground cover due to the association with white clover did not seem to influence aphid populations. As suggested by Finch and Kienegger (1997), the much smaller size of the white clover compared to wheat resulted in a dominance of the wheat in the top of the vegetation layer. The presence of a clover cover therefore did not significantly affect the accessibility of the host plant by the aphids.

#### 3.4.5 Grain yield and quality of wheat

The wheat grain yields in our study were not higher in the mixture of cultivars, which contradicts the prediction of the meta-analyses that showed a grain yield increase by 4.3% to 5.7% in winter wheat mixtures (Kiær et al., 2009; Borg et al., 2018). Such meta-analyses, however, confound cereal cultivar mixtures under organic and conventional management and have therefore to be taken with caution: because the crops do not grow under the same constraints, their performances are difficult to compare and so the general conclusion on the effect of cultivar mixtures is hardly reliable (Kaut et al., 2008). The absence of a significant difference in grain yield in our study also contrasts with former results obtained on organic barley. In that study, the heterogeneity in straw lengths resulted in an increased production (Kiær et al., 2012), which was not the case in our wheat mixture. It is possible that our two cultivars possess traits that are functionally redundant and so their mixing consequently would not increase the yields, as it has been observed in prior studies. Unfortunately, very little is known about the functional mechanisms resulting from heterogeneity and complementation of such traits in cultivar mixtures (Barot et al., 2017; Borg et al., 2018).

In the intercropped treatment, the wheat grain yield was reduced by 10%. This result is consistent with Thorsted et al. (2006a), but not with Vrignon-Brenas et al. (2018) who reported no significant difference in wheat yields among wheat monocultures and wheat

intercropped with white clover. Different conditions of competition, especially for water, may explain these contrasting results; in our study, interspecific competition between wheat and clover may have had an effect (Thorsted et al., 2006a). With the rainfall deficit in 2017, the intercropped wheat and clover may have competed belowground for water and mineral resources, affecting significantly the wheat's performance (Thorsted et al., 2006b).

Considering the loss in grain yield and the reduction in nitrogen content in our study, the trade-off between pest regulation and provisioning services may not be worth the costs and uncertainties associated with the implementation of these diversification practices. The range of the observed reduction of aphids in our study remains small. The economic injury level, above which an increase in aphid number causes economic damage, was found to be seven aphids or more per tiller for wheat aphids (Larsson, 2005), but in our fields, the mean aphid density never reached more than five aphids per tiller, and variation among treatments was below three aphids per tiller. In this context, the usefulness of the diversification practices to regulate aphids seems to be limited. Despite some promising studies that report interesting trade-offs between pest control and yields in polyculture systems using legumes as secondary crops (Iverson et al., 2014), no general conclusion on the performance of such system can be drawn. Indeed there are frequent variabilities among field studies, likely due to the multiple sources of interactions between biotic components and environmental conditions (Médiène et al., 2011)

### **3.5 Conclusion**

Our study showed that multi-level diversification in wheat fields did not result in either improved wheat production or increased aphid regulation. The combination of both genetic and species diversity resulted in non-additive effects on aphid regulation with yearly variation. Several reviews (Poveda et al., 2008; Ratnadass et al., 2012) have highlighted the difficulties in providing a straightforward effect of crop diversification on pests. If laboratory or semi-controlled small-scale experiments are essential to better understand the underlying mechanisms of pest regulation through increasing intrafield diversification, then our study confirms the necessity to realize this on large-scale farm studies. Environmental stressors may have favoured competition among plants instead of facilitation or complementation processes, thus producing contrasted results. We were subject to two contrasting climatic conditions in 2016 and 2017, which affected both aphid dynamics and canopy structure, although the latter may not have influenced aphid regulation. Integrating both the natural enemy response to combining two levels of crop diversification and taking into account landscape characteristics may provide valuable elements to characterize the potential of regulation of those practices (Jonsson et al., 2015).

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## **Chapter 4:**

Intercropping impacts the host location behaviour and population growth of aphids (Hemiptera: Aphididae)

This chapter corresponds to the article entitled **Intercropping impacts the host location behaviour and population growth of aphids (Hemiptera: Aphididae)** (Mansion-Vaquié et al.) submitted for publication in the journal *Entomologia Experimentalis et Applicata* in December 2018.

In the previous chapter, we observed that increasing interspecific diversity may influence cereal aphids, although the effects were variables according to the year and the plant growth. Laboratory tests enable us to study the behaviour of aphids towards intercropping under a controlled environment and without predators. In this chapter, we are therefore interested in the ecological process of "bottom-up control", which refers to the regulation of herbivores by the plants themselves (i.e. crop and non-crop). We aim at assessing the impact of intercropping on individual host location abilities and population growth of cereal aphids. As the response of arthropod pests to intercropping systems is relatively variable, we particularly seek to compare two different non-host plants in wheat-based intercrops and to determinate and discuss what parameters may be involved.

We remind the hypotheses addressed in this chapter:

- **Hypothesis 4:** aphid host location will be reduced in the presence of a non-host plant;
- **Hypothesis 5:** aphid population growth will be reduced in the presence of a non-host plant;
- **Hypothesis 6:** the negative effects of diversification on aphid host location and aphid population growth would differ according to the species used as non-host plant.

# Intercropping impacts the host location behaviour and population growth of aphids (Hemiptera: Aphididae)

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*Under review at Entomologia Experimentalis et Applicata*

**Abstract:** Increasing intrafield plant diversity has been shown to regulate pest populations in various agroecosystems. Among the suggested mechanisms for this bottom-up pest control, the disruptive crop hypothesis states that herbivores' abilities to locate and colonize their host plants are reduced by the presence of non-host plants. Under laboratory conditions, we evaluated how intercropping wheat and legumes modifies the behaviour of the cereal aphid *Sitobion avenae* (F.) (Hemiptera: Aphididea) in terms of host-plant location and population growth. We compared two intercropping systems—soft winter wheat (*Triticum aestivum* L.) associated with winter pea (*Pisum sativum* L.), or with white clover (*Trifolium repens* L.)—and sole stands of soft winter wheat. We observed that aphids needed more time to locate their host-plant of wheat and then spent less time on the wheat when it was intercropped with clover. At the population level, and if we take into account host plant biomass, only intercropping clover with wheat significantly reduced aphid densities on wheat. That is, intercropping clover with wheat was particularly disruptive to *S. avenae* behaviour and population growth. Our study points out that the species used as non-host plants and their density are important parameters that should be taken into account in studies on intercropping systems.

**Keywords:** Disruptive crop hypothesis, Bottom-up pest control, *Sitobion avenae*, Cereal, Legume

## **4.1 Introduction**

The link between biodiversity and ecosystem functioning has long been accepted (Naeem et al., 2002) and many experimental studies have showed that diversity is a key determinant of ecosystem processes, such as plant productivity (Tilman, 2015). Currently, a paradigm change is underway in agroecosystems management, with attempts to increase biodiversity at different spatio-temporal scales, from the crop field, to the farm, and finally across the landscape (Garibaldi et al., 2017). At the field level, increased diversity is mainly achieved by intercropping (Andow, 1991), defined as "*the cultivation of two or more species of crop in such a way that they interact agronomically*" (Vandermeer, 1989). We may distinguish two types of intercropping: "true intercropping" (Willey, 1979) that is the simultaneous cultivation of two cash crops, whereas "companion cropping" is the cultivation of a cash and a beneficial non-crop plant (Ben-Issa et al., 2017; Verret et al., 2017). An increase in productivity in intercropping systems may be achieved through an improved yield per unit area, namely due to complementary use of resources, facilitation, and/or increased pest regulation (Brooker et al., 2015).

In this study, we are interested in the role of intercropping on the control of aphids (Hemiptera: Aphididae), which are one of the main pests in temperate regions (Dedryver et al., 2010). We focus on cereals and legumes because cereals are important in most temperate farming systems and are dominant crops in terms of agricultural area, and increasing the cultivation of legumes for animal feed or for food and other ecosystem services (e.g., supply of nitrogen) is becoming central in agriculture (Stagnari et al., 2017). Intercropping cereals and legumes is considered to have several agronomical and environmental advantages (Bedoussac & Justes, 2010a; Lithourgidis et al., 2011; Pelzer et al., 2012; Bedoussac et al., 2015), including the reduction in numbers of cereal and legume aphids (Ndzana et al., 2014; Lopes et al., 2015; Hatt et al., 2018; Xu et al., 2018). However, the ecological mechanisms at the origin of these changes in abundance are still under discussion.

Plant diversity is believed to promote pest regulation due to two main ecological processes: bottom-up and top-down effects, depending on whether the control of herbivores is driven respectively by lower or upper trophic levels (Gurr et al., 2017). In the case of aphids, control seems to depend on both, with top-down effects of the trophic functional groups of natural enemies (specialists or generalists) being modulated by interactions between aphids and their host plant (Diehl et al., 2013). Several hypotheses have been put forward to explain how increasing intrafield diversity may promote bottom-up control. The disruptive crop hypothesis suggests that the presence of a non-host plant would lower the targeted herbivore's ability to locate and colonize its host plant (Vandermeer, 1989; Finch & Collier, 2000; Poveda et al., 2008). One of the most important ways in which aphids find and select a host plant is by using chemical cues (Webster, 2012; Döring, 2014), and so a non-host plant may interfere with the chemical cues by masking the host's odours, altering the host's chemical profile, or introducing repellent compounds (Finch & Collier, 2000; Randlkofer et al., 2010; Xie et al., 2012; Ninkovic et al., 2013; Ben-Issa et al., 2017). While the interference of chemical cues has been the most studied hypothesis, other hypotheses have been put forward, namely the "physical obstruction" or "barrier crop" hypotheses (Perrin & Phillips, 1978), and the "visual camouflage" hypothesis (Finch & Collier, 2000), which suggest that pests face increasing difficulties in locating their host plant respectively due to a more arduous physical access to it or because the host plant is hidden. That is, a more complex architecture of the vegetation would disrupt the herbivores behaviour and prevent them from easily reaching their host plant.

Although aphid species initially infest annual crops through the migration of flying morphs from surrounding habitats (Fievet et al., 2007; Irwin et al., 2007), later generations mainly comprise wingless individuals. Walking represents an essential and frequent mode of transport during an aphid's life-time (Irwin et al., 2007): when the competition in a colony increases, local dispersion, such as intra- or inter-plant movements, acquires great importance



and affects the aphids' colonization process (Lombaert et al., 2006). For instance, in an alfalfa field experiment, the majority of apterous pea aphids could walk up to 2 m in a time frame of 4 h (Ben-Ari et al., 2015). Although walking plays a determining role in the spread of the infestation at the field scale (Hodgson, 1991), on the individual aphid scale, leaving a plant to look for another one is both risky and energy-costly. Without a doubt, this carries fitness costs, and thus trade-offs between foraging and reproductive success are common (Stearns, 1992). Indeed, in aphids the different dispersal strategies between alate and apterous individuals correspond to different sizes of gonads, with alate aphids being less fecund than their apterous counterparts (Dixon et al., 1993; Braendle et al., 2006). Moreover, apterous pea aphids that had dropped off or walked away from their host plant to avoid predators were shown to have reduced offspring, with consequences for population growth, compared to apterous aphids undisturbed by predators (Nelson et al., 2004).

In this study, we compared the host location behaviour and population growth of individuals of the cereal aphid *Sitobion avenae* (F.) in two intercropping and one mono-cropping systems: soft winter wheat (*Triticum aestivum* L.)-winter pea (*Pisum sativum* L.), soft winter wheat-white clover (*Trifolium repens* L.), and pure stands of soft winter wheat. First, we hypothesized that aphids' host plant location is reduced in the presence of a non-host plant, and we conducted a short-time behavioural experiment to evaluate it. Secondly, using a long-term experiment, we tested the hypothesis that aphid population growth is limited in the presence of a non-host plant. In this experiment, we also considered that intercropping may affect plant quality, and in turn may affect aphid performance, which is determined by sap quality especially in terms of nitrogen content from free amino acids (Dinant et al., 2010). Finally, we were interested in comparing two different non-host plants that could be used in wheat-based intercropping systems, namely winter pea and white clover (Lopes et al., 2016; Vrignon-Brenas et al., 2018). Those plants are structurally different: winter pea plants are characterized by an indeterminate growth (>60 cm) (Cousin, 1997; Bedoussac & Justes,

2010b) and often produce tendrils that enhance connectivity between plants, while white clover develops horizontally and does not reach tall heights (20-30 cm) (Frame & Newbould, 1986; Frame, 2005). Furthermore, in field conditions these non-host plants are seeded in different densities.

## **4.2 Material and methods**

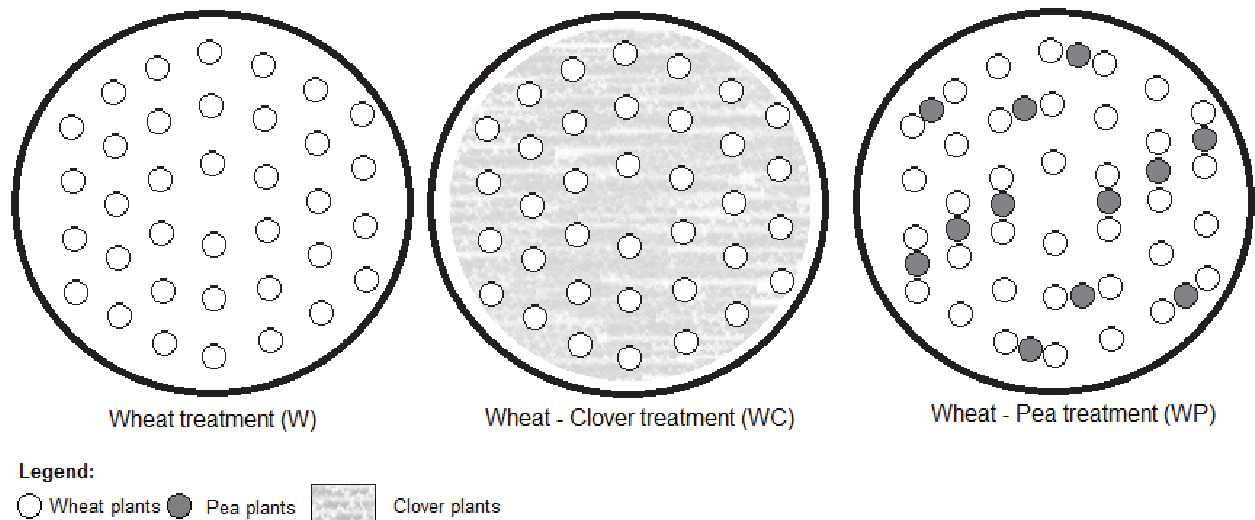
### **4.2.1 Aphid stock cultures**

A *S. avenae* aphid stock culture was initiated in the laboratory using individuals obtained from Koppert, The Netherlands (Ervibank©). The colony was maintained on winter wheat grown in 2 L pots in an insect-free chamber controlled for light and temperature (L16:D8; 21°C).

### **4.2.2 Experimental plants**

Plants used in the experiments were grown from organic seeds following three experimental treatments: a wheat monoculture treatment (W), and two intercropped treatments, wheat-winter pea (WP) and wheat-white clover (WC) (Figure 4.1). For all experiments, winter wheat (*Triticum aestivum* L. cv Renan) was grown in 4 L pots (diam. 18cm, height 15cm) containing soil substrate (®Jiffy substrates NFU 44-551) in an insect-free chamber controlled for light and temperature (L16:D8; 21°C) and watered with 500 mL at seeding and 250 mL every two or three days. There were 36 wheat seeds sown per pot in a systematic design where seeds were 2 cm distance from each other. Winter pea (*Pisum sativum* cv Enduro) was sown with wheat in an additive design by inserting 1 pea seed every 3 wheat seeds for a total of 12 pea seeds per pot. White clover (*Trifolium repens* cv Rivendel) was homogeneously sown (70 mg, i.e., ±100 seeds) in an additive design. All seeds were free of pesticides treatments. Those seed densities correspond to the ones used in the field (Ndzana et al., 2014; Vrignon-Brenas et al., 2018). In the few cases where some seeds of wheat or pea did not germinate after five to six

days, they were replaced with plants grown under the same conditions in extra pots. We counted 85 grown clover plants on average per experimental pot. Plants used in the experiments were seven days old.



**Figure 4.1: Schematic representation of the pots experimental design.**

#### 4.2.3 Behavioural experiment

In order to investigate the aphids' behaviour in the different treatments (W, WP or WC), we ran an experiment with five sessions, with ten pots of each treatment per session. Before starting, the vegetation cover was characterized for each pot. We measured the height of five plants of each species and the number of contacts among plants (i.e., among wheat plants and between wheat and associated plants). Additionally, we calculated the percentage of total ground area covered by vegetation: each experimental pot was photographed from above and the photographs were eventually analysed using the ImageJ software, version 1.44o (National Institute of Health, USA). The experiment took place in an insect-free chamber controlled for light and temperature (L16:D8; 21°C).

Experimental aphids were standardized for age prior to the experiments: adult aphids were individually placed in a tube of glass (Ø 20 mm, height 15-20 cm) on a plant of wheat that was seven days old, previously transplanted from soil to a wet cotton bed. After 48 h, the

adult aphid was removed and the newborn nymphs were kept in the tube for seven to eight days until they became adults. They were then transferred to an insect-free chamber controlled for light and temperature (L16:D8; 21°C), where the experiment took place.

Aphids were starved for 3 h before the beginning of the experiment to reduce the variability due to individual physiological conditions (Quiroz et al., 1992; Caillaud & Via, 2000). Then, using a fine brush, each aphid was carefully placed on the ground at the centre of a pot, and once the aphids started to move, the time spent on the wheat or the associated non-host plant or the ground was recorded continuously for five minutes. Additionally, the position of the aphid was recorded at 10 min, 20 min, 30 min, and 1 h after the beginning of the experiment. Due to the small size of the clover, it was difficult to follow the movement between clover plants without disturbing the experiment; therefore only visits on the clover cover were considered. Whenever the aphid reached the edge of the pot, the observation stopped and the aphid was considered to be escaping and so was removed from the experiment. A total of 149 aphids were monitored.

#### 4.2.4 Aphid population growth experiment

In order to investigate the aphid population growth in the different treatments, we used 66 experimental pots per treatment (i.e., 198 experimental pots in total). On the first day of the experiment and for each treatment, half of the experimental pots were inoculated each with five adult and five nymph wingless aphids from the stock cultures; the remaining pots were the non-infested controls. All pots were enclosed in a plastic frame (37 cm high; Ø 20.5 cm) covered with a tight meshed net, which prevented the aphids from either escaping from the infested pots or entering the control pots. Then, 7, 14, and 21 days after the inoculation, 22 pots of each treatment (11 infested and 11 control) were randomly removed from the experiment. In each infested pot, aphids were manually collected from the plant and placed in a Petri dish (Ø 55 mm), which was then kept at -80°C. Later, the number of winged and wingless adults and nymphs were counted under a stereoscopic microscope.

The wheat above-ground biomass was collected, dried at 60°C for 48 h and weighed using a portable laboratory scale balance (PT210-000V1, Sartorius®), except 2.5 g of the fresh wheat biomass, that was kept aside at -20°C for chemical analyses in order to measure the concentration of free amino acids in the wheat leaves. These chemical analyses were performed as follows: the wheat leaves were dried in a vacuum oven (40°C and -1bar) for 36 h and then shredded with a ball mill, and then 20 mg of this wheat leaf powder was diluted in 500 mL distilled water and heated at 70°C for one hour. After cooling, the solution was centrifuged for 5 min at 10.000 rpm and the resulting supernatant was analysed with the kit K-PANOPA© (Megazymes, Ireland), in accordance to the manufacturer's protocol.

#### 4.2.5 Statistical analysis

Concerning the behavioural experiment, data was analysed using mixed models (Table 4.1), with *experimental session* as a random factor to take into account dependency of observations of experimental pots made at a same date. General linear mixed models (GLMMs) and Linear mixed models (LMMs) were built respectively with the *glmer* and *lmer* functions from the *lme4* package (Bates et al., 2015).

The significance of fixed factors and their interaction was determined by a F-test with a Kenward-Roger correction for LMMs or likelihood ratio tests (LRT) for GLMMs, as implemented in the *mixed* function in the *afex* package (Singmann et al., 2018). If non-significant, the interaction was removed from the model and the significance of fixed factors was retested. The time that the aphids needed to reach their first wheat plant was compared among treatments with a survival time analysis using the Log-rank test from the *survfit* function in the *survival* package (Therneau & Lumley, 2017).

**Table 4.1: Presentation of the statistical analyses performed for each studied variable in the behavioural experiment and the explanatory fixed factors and random factors when applicable.**

<b>Variables</b>	<b>Statistical analyses</b>	<b>Fixed factors</b>	<b>Random factors</b>
% aphids leaving the first plant encountered	Binomial GLMM	<i>Treatment and Identity of first plant encountered</i>	
N visits of plants or ground			
N escaped aphids	Poisson GLMM	<i>Treatment</i>	<i>Experimental session</i>
N contacts among plants			
Time spent on plants			
Vegetation cover	LMM	<i>Treatment</i>	
Wheat height			
Time needed to reach the first wheat plant	Log-rank test	<i>Treatment</i>	NA
Probability to encounter wheat first	Exact binomial test	NA	NA

Concerning the population growth experiment (Table 4.2), aphid numbers were compared with a quasiPoisson GLM to account for overdispersion. When non-significant, the interaction was removed from the model and the significance of fixed factors was retested. Aphid densities (number of aphids over plant weight) were analysed with a non-linear quadratic regression. The demographic composition of the aphid populations at single dates was analysed with ANOVA, or with Kruskal-Wallis rank sum test when data were not normally distributed. For the ANOVA, all possible interactions were considered and non-significant interactions were removed using a stepwise model selection by AIC. For all models and when appropriate, pairwise comparisons were realized using Tukey-adjusted least-square means (LSMeans) with the *emmeans* package (Lenth, 2018). All statistical analyses were conducted using R software, version 3.4.3 (R Development Core Team, 2017).

**Table 4.2: Presentation of the statistical analyses performed for each studied variable in the aphid population growth experiment and the explanatory fixed factors.**

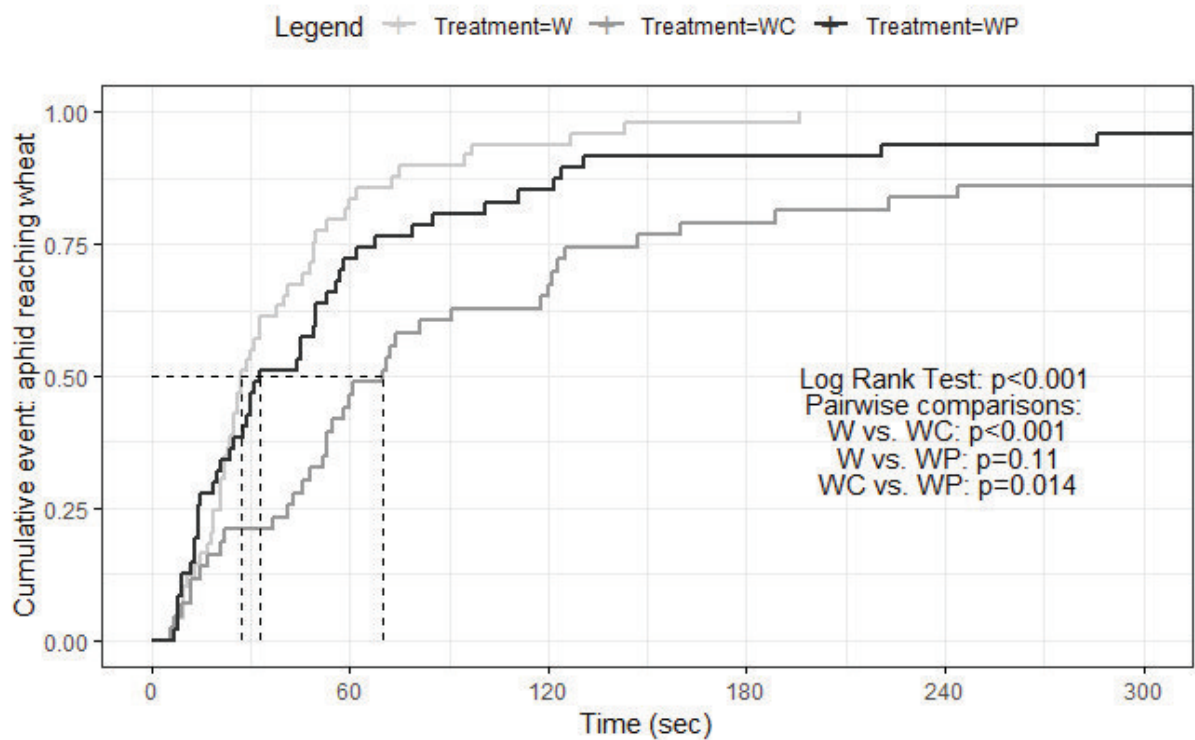
<b>Variables</b>	<b>Statistical analyses</b>	<b>Fixed factors</b>
Aphid abundance	quasiPoisson GLM	<i>Treatment and Days</i>
Aphid densities	Quadratic (non-linear) regression	<i>Treatment and Days</i>
Adult aphid densities	ANOVA	<i>Treatment</i>
Winged aphid densities	Kruskall-Wallis rank sum test	<i>Treatment</i>
Wheat dry biomass	ANOVA	<i>Treatment, Aphid (presence or absence) and Days</i>
Free amino acid concentration		<i>Treatment and Days</i>

## **4.3 Results**

### **4.3.1 Behavioural experiment**

Due to the difference in seeding densities, the theoretical probability that an aphid first encountered a wheat plant was 0.75 for WP, 0.30 for WC, and obviously 1.00 for W. Our observations did not significantly differ from this (Table 4.3): 66% of aphids first visited a wheat plant in WP (Exact binomial test:  $CI_{95\%} = 0.51 - 0.79$ , P-value = 0.14), while only 30% of aphids first visited a wheat plant in WC (Exact binomial test:  $CI_{95\%} = 0.18 - 0.45$ , P-value = 1.00). All aphids reached a wheat plant in W.

Furthermore, the time needed to reach a wheat plant was significantly different among treatments (Figure 4.2; Log Rank Test:  $X^2 = 17.9$ , d.f.= 2, P-value < 0.001): it was longer for aphids in WC (70 s) compared to those in the WP and W treatments, which took similar times (33 s and 27 s, respectively).



**Figure 4.2:** Kaplan Meier plot of the time needed for aphids to reach a wheat plant during the first five minutes of the experiment according to the treatments. W: Wheat monoculture, WC: Wheat intercropped with clover, WP: Wheat intercropped with pea.

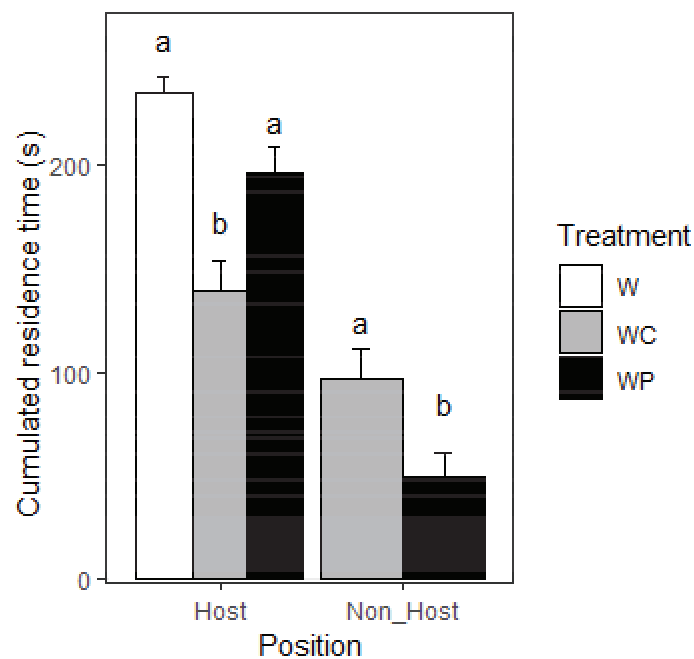
All 149 aphids except one visited at least one plant during the first five minutes of the experiment, but more than 50% of the aphids left the first plant that they visited during that period (Table 4.3). The probability of leaving the first plant visited was greater when the plant was a non-host plant (LRT on GLMM:  $X^2 = 5.60$ , d.f.= 1, P-value =0.02) and this probability was similar across treatments (LRT on GLMM:  $X^2 = 0.37$ , d.f.= 2, P-value =0.83).

**Table 4.3:** Behaviour of aphids concerning the first plant encountered according to the treatments W: Wheat monoculture, WC: Wheat associated with clover, WP: Wheat associated with pea) and the identity of the first plant encountered (host or non-host). Leave refers to the aphids walking away from the first plant encountered; Stay refers to the aphids remaining on the first plant encountered.

Treatment:	W (n=49)	WP (n=50)		WC (n=50)			
First plant visited	Wheat	Wheat	Pea	Wheat	Clover	None	
	100%	66%	34%	28%	70%	2%	
Behaviour	Leave	57%	34%	26%	16%	56%	na
	Stay	43%	32%	8%	12%	14%	na



During the first five minutes, aphids came back to the ground more often in WC (1.3 times on average) compared to the other treatments with less than one return on average (LRT on GLMM:  $X^2 = 14.69$ , d.f. = 2, P-value < 0.001). Aphids visited an equivalent number of wheat plants (1.5 on average) whatever the treatment (LRT on GLMM:  $X^2 = 3.43$ , d.f. = 3, P-value = 0.33) but nevertheless spent significantly more time on wheat in the W (234 s) and WP (195 s) treatments compared to the WC (139 s) treatment (Figure 4.3; F-test on LMM:  $F_{2,140} = 14.5$ , P-value < 0.001). Concerning the non-host plants (i.e., clover or pea), aphids spent significantly more time on clover (97 s) compared to pea (49 s) (F-test on LMM:  $F_{1,92} = 6.9$ , P-value = 0.01).



**Figure 4.3:** Barplots of the cumulated residence time in seconds (mean +S.E.) of aphids on wheat host plants and on non-host plant and during the first five minutes of the experiment according to the treatments. W: Wheat monoculture, WC: Wheat intercropped with clover, WP: Wheat intercropped with pea. Tukey-adjusted pairwise LSM means comparisons (P-value < 0.05).

During the 1 h experiment, 27% aphids left the experimental pot; the rate of escape was not significantly different among treatments (LRT on GLMM:  $X^2 = 0.12$ , d.f. = 2, P-value = 0.94).

For those who remained in the experimental pots, 94% and 88% visited at least one wheat plant respectively in the WP and WC treatments ( $X^2 = 0.49$ , d.f. = 1, P-value = 0.49).

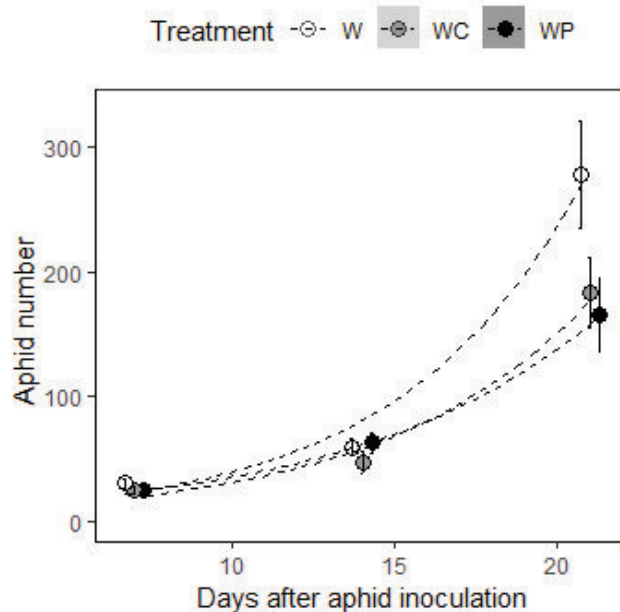
Differences in aphid behaviour according to the treatments may be related to contrasting vegetation covers. Characteristics of the vegetation are described for each treatment in Table 4.4. As expected from seeding densities, ground cover was significantly different among treatments (F-test on LMM:  $F = 17.65$ , d.f. = 1, P-value < 0.001): it was largest for the treatment WC, intermediate for WP and lowest for wheat in monoculture. Pea plants never touched each other, while clover plants formed a continuous cover such that it was difficult to distinguish every single contact among plants. Wheat plants were significantly more in contact with clover than with pea plants (Table 4.4).

**Table 4.4: Vegetation characteristics: ground cover, contacts between wheat plants and between wheat and non-host plants (clover and pea), and plant heights according to the different treatments (means  $\pm$  SEM). Different letters indicate significant differences among treatments for a specific variable according to Tukey-adjusted pairwise LSM means comparisons ( $P < 0.05$ ) applied on LMMs for vegetation cover and wheat height or GLMMs for contacts among plants.**

	Treatments		
	Wheat	Wheat-Clover	Wheat-Pea
Ground Cover (%)	13.4 $\pm$ 0.4 (a)	16.5 $\pm$ 0.4 (b)	14.8 $\pm$ 0.4 (c)
Contacts among wheat plants	6.0 $\pm$ 0.5 (a)	6.8 $\pm$ 0.4 (a)	4.4 $\pm$ 0.4 (b)
Contacts between wheat and non-host plants	-	6.9 $\pm$ 0.4 (a)	2.3 $\pm$ 0.2 (b)
Wheat height (cm)	13.4 $\pm$ 0.2 (a)	13.6 $\pm$ 0.2 (a)	12.7 $\pm$ 0.2 (b)
Non-host plant height (cm)	-	1.4 $\pm$ 0.0	4.3 $\pm$ 0.1

### 4.3.2 Aphid population growth experiment

Aphid populations significantly increased over the 3-week experiment (Figure 4.4) (GLM:  $F_{1,91} = 223.97$ , P-value < 0.001) and aphid number was significantly different among treatments (GLM:  $F_{2,92} = 5.68$ , P-value < 0.01): intercropping wheat with a legume significantly reduced the number of aphids compared to sole wheat, but no difference between WC and WP was observed (Tukey-adjusted pairwise LSMMeans comparisons with P-value < 0.05). We did observe, however, differences among wheat biomasses from different treatments at 21 days of infestation (Figure 4.5): the biomass of wheat associated with pea was significantly lower when compared to wheat in monoculture and to wheat associated with clover. Though there were differences among treatments, the biomasses of wheat infested with aphids were significantly lower compared to the control plants.



**Figure 4.4:** Aphid number (mean  $\pm$ S.E.) over 3 weeks after aphid inoculation according to the treatments W: Wheat monoculture, WC: Wheat intercropped with clover, WP: Wheat intercropped with pea. Dashed lines result from the quasisoisson GLM analysis that best described the data.

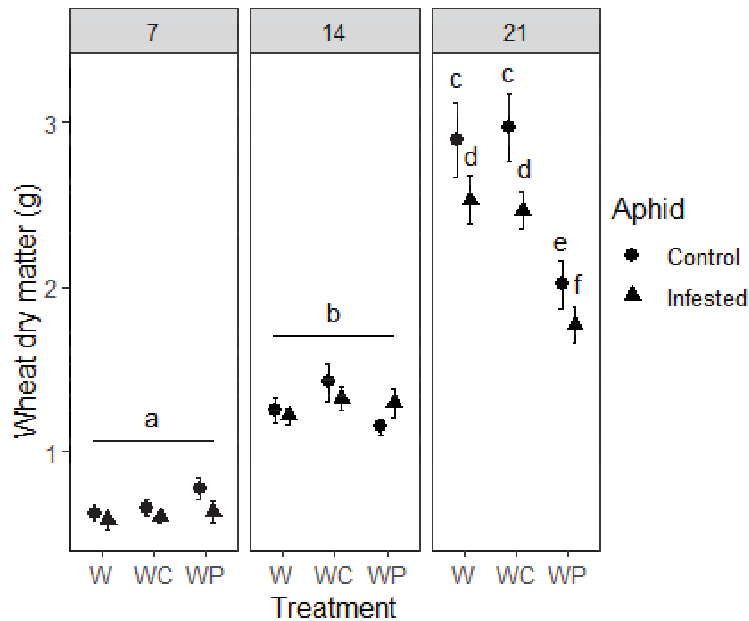


Figure 4.5: Wheat dry biomasses (mean  $\pm$  S.E.) over 3 weeks after aphid inoculation according to the treatments and according to the presence (infested) or absence (control) of aphids. W: Wheat monoculture, WC: Wheat intercropped with clover, WP: Wheat intercropped with pea. Different letters indicate significant differences according to Tukey-adjusted pairwise LSMeans comparisons (P-value < 0.05).

Therefore, if we take into account aphid densities, i.e., number of aphids per gram of dry wheat mass (Figure 4.6), it can be seen that intercropping wheat with clover reduced aphid densities compared to sole wheat and to wheat intercropped with pea (Tukey-adjusted pairwise LSMeans comparisons with P-value < 0.05).

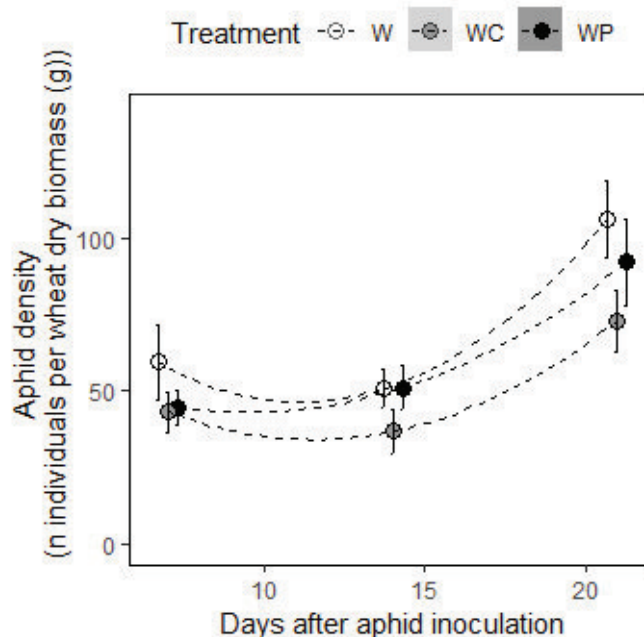


Figure 4.6: Aphid densities (mean  $\pm$  S.E.) over 3 weeks after aphid inoculation according to the treatments W: Wheat monoculture, WC: Wheat intercropped with clover, WP: Wheat intercropped with pea. Dashed lines result from the quadratic regression analysis that best described the data.

The demographic composition of the aphid populations in each experimental treatment and for each sampling date (7, 14 and 21 days) is described in Table 4.5 (number of aphids over wheat biomass). Aphid populations were dominated by apterous nymphs, which drove the overall results presented above. The density of winged aphids (adults and nymphs) was negligible at the first two sampling dates and still represented less than 2% of the aphid population at 21 days. At this last sampling date, the mean density of winged aphids was not significantly different between treatments (Kruskall-Wallis rank sum test:  $X^2 = 0.66$ , d.f. = 2, P-value = 0.72). However, it should be noted that winged aphids were present in the WC treatment since the first sampling date, contrary to the other treatments where they only appeared in the 21 days sampling.

**Table 4.5: Densities (number of aphids over plant weight; mean  $\pm$  S.E.) of apterous and winged aphids according to their growth stage and the treatments: Wheat monoculture, Wheat associated with clover, Wheat associated with pea and at 7, 14 and 21 days after infestation.**

		Treatments			
		Wheat	Wheat-Clover	Wheat-Pea	
<b>7 days</b>	Apterous	Adults	2.0 $\pm$ 0.5	1.2 $\pm$ 0.5	1.4 $\pm$ 0.4
		Nymphs	57.4 $\pm$ 12.3	41.8 $\pm$ 6.3	43.3 $\pm$ 5.7
	Winged	Adults	0	0.3 $\pm$ 0.2	0
		Nymphs	0	0	0
<b>14 days</b>	Apterous	Adults	7.3 $\pm$ 1.3	5.1 $\pm$ 0.9	9.8 $\pm$ 1.3
		Nymphs	43.3 $\pm$ 5.6	31.6 $\pm$ 6.7	41.3 $\pm$ 6.1
	Winged	Adults	0	0.1 $\pm$ 0.1	0
		Nymphs	0	0	0
<b>21 days</b>	Apterous	Adults	14.0 $\pm$ 1.8	10.8 $\pm$ 1.6	12.1 $\pm$ 2.2
		Nymphs	90.7 $\pm$ 10.9	61.1 $\pm$ 8.3	79.1 $\pm$ 12.2
	Winged	Adults	0.3 $\pm$ 0.1	0.4 $\pm$ 0.3	0.1 $\pm$ 0.1
		Nymphs	1.2 $\pm$ 0.5	0.8 $\pm$ 0.3	0.9 $\pm$ 0.4

In order to seek a possible relationship between aphid population growth in the different treatments and plant sap quality when intercropped, we analysed the concentration in free amino acids in the wheat leaves of the non-infested plants (i.e., controls) of the different treatments (Figure 4.7). Free amino acids significantly decreased over time (ANOVA:  $F_{2,87} = 63.07$ , P-value < 0.001) and their concentration was not different among treatments (ANOVA:  $F_{2,87} = 1.60$ , P-value = 0.57). There was a significant interaction between time and treatment (ANOVA:  $F_{4,87} = 7.56$ , P-value = 0.04) due to the significant difference in free amino acid concentration in wheat plants from W and WP treatments 14 days after aphid infestation, but this difference disappeared later on.

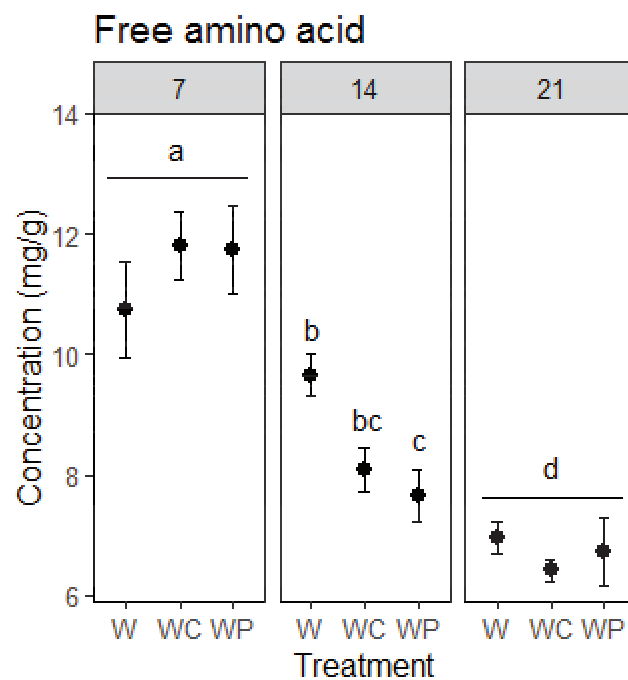


Figure 4.7: Free amino acid concentration (mg/g) in wheat leaves of control plants (mean  $\pm$ S.E.) over 3 weeks according to the treatments. W: Wheat monoculture, WC: Wheat intercropped with clover, WP: Wheat intercropped with pea. Different letters indicate significant differences according to Tukey-adjusted pairwise LSM means comparisons (P-value < 0.05).

#### **4.4 Discussion**

The present study aimed at verifying the hypotheses that combining wheat and legumes modifies the behaviour of the cereal aphid *S. avenae* in terms of host-plant location and population growth. We observed that the aphids' residence time on wheat decreased when this host-plant was intercropped with clover. At the population level, wheat-legume intercrops reduced the number of aphids on wheat plants compared to wheat sole crops, but if we take into account plant biomass, only intercropping clover with wheat significantly reduced aphid densities on wheat. That is to say, intercropping clover with wheat was particularly disruptive to *S. avenae* behaviour.

Our observations support our first hypothesis that host location by aphids was impeded by the presence of a non-host plant. The results seem to be driven mostly by differences in plant densities, which corresponded in our experiment to an additive intercrop design simulating field seed densities of wheat-pea and wheat-clover intercrops. Although connectivity between plants was higher in the wheat-clover treatment, this did not help aphids to more easily reach their host plant. Blurred olfactory signals as well as visual barrier effects may explain this result, because they are both related to non-host plant density and are involved in host location (Gish & Inbar, 2006; Reeves, 2011; Webster, 2012).

The experiments of Xie et al. (2012) and Ninkovic et al. (2013) showed that both alate and apterous aphids demonstrate a preference for the volatile organic compounds emitted by pure stands of wheat compared to those emitted by intercrops. Odours therefore play a role as first attractors to host plants. However, the rejection of a non-host plant by insects occurs after reaching the plants, not before (Finch & Collier, 2012). Once on the plant, aphids initiate a second recognition process with their antennae by probing the plant to obtain physical and chemical information about its suitability (Caillaud & Via, 2000). This recognition stage takes around 60 seconds and is essential to avoid full stylet penetration on the wrong plant, which is

time consuming (45 min, Dixon, 1998). Therefore, in case of high non-host plant density, this second stage has to be repeated for each encountered plant, increasing the time necessary to ultimately come upon the host-plant. Indeed, in our experiment we observed that intercropping wheat with clover increased the aphids' movements between the ground and the clover cover and within the clover cover, significantly delaying the aphids' arrival to the wheat. Clover, at least in the tested growth stage and experimental seed density, represents an ideal plant barrier, as defined by Hooks & Fereres (2006), because it allows the aphid sufficient residence time for probing before taking-off occurs. These observations imply that the ratio of the two intercropped plants may be extremely important for pest control.

Concerning our second hypothesis, the results of the experiment on aphid population growth suggest a bottom-up regulation through plant interspecific diversification. However, intercropping can reduce intercropped plant mass (Thorsted et al., 2006b), and confounding effects from interspecific competition between wheat and non-host plants may be misleading (Bukovinszky et al., 2004). Therefore, biomass losses should be taken into account when considering intercrop infestation effects (e.g., Ndzana et al., 2014). This means that in our case, *S. avenae* infestation is only significantly lower for the wheat-clover intercrop treatment.

The lower number of aphids in wheat-clover intercrops can be explained by a longer female developmental time, decreased fecundity, increased mortality, and/or a higher level of emigration. Emigration can be discarded in our experimental set because each pot was isolated under a net. However, in the wheat-clover treatment alate aphids appeared since the first week of infestation, indicating that migration was induced and thus could have strengthened the decrease in aphid numbers if winged aphids were allowed to leave the experiment.

All the above-mentioned processes could be influenced indirectly by the effect of cover structure on aphids' behaviour and/or directly by plant quality. On the one hand, the increased walking activity on wheat-clover treatments, observed in our first experiment, could carry



fitness costs which are known to trade-off with reproductive success (Stearns, 1992) with consequences for population growth (Hooks & Fereres, 2006). Moreover, repetitive probing of a non-host plant associated with failure in host location is known to induce flight in aphids (Finch & Collier, 2000). On the other hand, cereals and legumes compete for soil nutrients at early growth stages (Corre-Hellou et al., 2006; Bedoussac et al., 2015). Such competition may impact plant quality and constrain the growth rate of aphids (Theunissen, 1994; Dixon, 1998) and induce the production of winged aphids to escape unsuitable conditions (Braendle et al., 2006; Awmack & Leather, 2007; Irwin et al., 2007). Nevertheless, in our experiment, we did not find either a decrease in the biomass or differences in the concentration of free amino acids in the leaves of wheat intercropped with clover compared to those from wheat monocultures. Unfortunately, our method does not distinguish essential and unessential amino acids, which together with the fact that the pathways for the uptake and utilization of host plant nutrients by aphids are still under discussion (Vogel & Moran, 2011; Haribal & Jander, 2015), makes it difficult to have a clear picture of the link between sap quality and aphid population growth. Finally, our results only concern the first four weeks of plant growth, while *S. avenae* mainly colonizes later wheat developmental stages. Although this aphid species reacts to the nutritional quality of the host plant associated to seed ripening (Walters & Dixon, 1982), a growth stage where clover reduces wheat above ground biomass (Thorsted et al., 2006b), nothing is known about the influence of the nutritional quality of early plant stages on its development.

## **4.5 Conclusion**

Flight arrival of aphids on a crop has been shown to decrease with increased vegetation cover and diversity (Finch & Collier, 2000; Döring, 2014). Although it has been argued that increasing vegetation diversity may be more effective in reducing the winged aphid colonizers of wheat host plants than in regulating the later spread of aphid colonies (Costello & Altieri, 1995; Wratten et al., 2007), our study shows that vegetation diversity can also effectively contribute to decreasing the growth of the later wingless aphid populations. Nevertheless, this depends on the species used as non-host plants, and their density. Although additive versus substitutive designs are often compared in studies concerning the effect of intercropping on pest control, little is reported about the influence of plant relative densities on pest dispersal (Lopes et al., 2016). Our study points out that these are important parameters that should be taken into account. In our experimental set that copied additive designs used in real farming conditions, intercropping clover with wheat was particularly disruptive to *S. avenae* and may be an interesting strategy for its control. Nevertheless, our observations are valid only for aphid colonization of early plant growth stages. Later on, the vegetation structure as well as interspecific interactions (namely competition for nutrients) may change and differently influence aphid behaviour, and this should be further investigated.

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### **Author Contributions**

Conceived and designed the experiments: AMV, AM & AF. Performed the behavioural experiments: AMV & FRP. Performed the aphid population experiments: AMV & AM. Did the chemical and the photo analyses: FRP. Analyzed the data: AMV. Wrote the paper: AMV, AM, AF & AW.

## **Chapter 5:**

Combining intraspecific and interspecific crop  
diversification for improved conservation biological  
control in wheat fields

In the previous chapter, we were particularly interested in the ecological process of "bottom-up control" promoted by intercropping. In the fields, another ecological process may also be involved in the control of pests by increasing the intrafield plant diversity: the "top-down control", which refers to the regulation of herbivores by their natural enemies. Similarly to the Chapter 3, we aim at investigating the influence of intraspecific and interspecific diversification practices, and their combination, on the populations of aphids' natural enemies in wheat fields. As the presence only of natural enemies is not a guarantee of their predation activity on the pest, we also aim at assessing the predation pressure they may exert on the pests in the fields through the use of sentinel preys.

We remind the hypotheses addressed in this chapter:

- **Hypothesis 7:** natural enemies' abundance and diversity will be increased by each single diversification practice;
- **Hypothesis 8:** natural enemies' abundance and diversity will be increased in a synergistic way by the combination of both diversification practices;
- **Hypothesis 9:** predation activity and parasitism rates will be increased by each single diversification practice;
- **Hypothesis 10:** predation activity and parasitism rates will be increased by the combination of both diversification practices.

# **Combining intraspecific and interspecific crop diversification for improved conservation biological control in wheat fields**

Agathe MANSION-VAQUIÉ, Vincent TOLON, Alexander WEZEL & Aurélie FERRER

*In preparation for submission*

## **Abstract:**

The effectiveness of natural enemies to control pest can be enhanced through habitat manipulation that aims to meet the ecological requirements of beneficial arthropods in terms of habitat and food sources. In this study we assessed how increasing genetic plant diversity (wheat cultivar mixture), species plant diversity (intercropping winter wheat and white clover), and the combination of both diversity, influence the community of predatory arthropods and their role as biological control agents. On field experiments over two growing seasons, we monitored foliage and ground dwelling predatory arthropods through visual counting and pitfall trapping, respectively. We also assessed the parasitism rate and we measured the predation pressure of natural enemies using two different sentinel prey methods: artificial caterpillars at the ground level and aphid predation cards at the foliage level. We observed that natural enemies, especially spiders and ground beetles, tended to respond positively to the presence of a clover cover in the wheat fields, but did not respond to the wheat cultivar mixture. Results varied according to the different taxa of arthropods concerned and their spatial occurrence in the vegetation layers (ground dwelling or foliage dwelling arthropods). The community composition of predatory ground dwelling beetles was influenced by the cover of clover, with different effects according to the fields. Neither the rates of predation on sentinel preys nor the parasitism rate were influenced by any of the diversification practices. The combination of both diversification practices did not outperform each practice individually in attracting natural enemies nor increasing their predation activity.

**Keywords:** Conservation biological control; Cultivar mixtures; Cover crop; Ground beetles, Sentinel prey; Organic agriculture

## **5.1 Introduction**

In the search for more sustainable farming practices, the development of biodiversity-based agriculture aims to reduce the use of external inputs and optimize naturally occurring ecological processes (Duru et al., 2015). As an alternative to pesticides, habitat management refers to vegetation manipulation with the intended consequence of suppressing pest densities (Gurr et al., 2017). In this context, increasing intrafield plant diversity has been shown to regulate pest populations in various agroecosystems, with variable results according to arthropod species and cropping systems (Letourneau et al., 2011; Dassou & Tixier, 2016; Lopes et al., 2016). Such pest regulation may be a result of more abundant communities of natural enemies (Symondson et al., 2002; Gurr et al., 2017). Diversified environments are indeed expected to sustain the presence and the activity of natural enemies (natural enemy hypothesis; Root, 1973) due to the provision of shelter, nectar, alternative prey/hosts, and pollen (Gurr et al., 2017). Habitat management is therefore an important component of conservation biological control (Gurr et al., 2017).

At the field level, increased plant diversity is mainly achieved by increasing the number of crop species (interspecific diversity) such as the combination of two crops (intercropping) or a crop and a beneficial non-crop plant (companion crop) (Andow, 1991; Ben-Issa et al., 2017). But it can also be implemented at the intraspecific level by mixing different varieties of a crop species (polyvarietal mixtures) (Andow, 1991; Koricheva & Hayes, 2018). Successes in promoting natural enemies have been reported for both diversification practices. Interspecific diversity increased abundance of both ground dwelling natural enemies such as spiders, carabids and staphylinids (Kromp, 1999; Sunderland & Samu, 2000; Gravesen, 2008), and foliage dwelling predators, including lady beetles, syrphids and parasitoids (Wang et al., 2009; Seidenglanz et al., 2011; Xie et al., 2012). Especially

polycultures including a higher ground cover through cover crops or living mulches may benefit natural enemies by providing attractive microclimate and shelter within crop fields (Booij et al., 1997; Carmona & Landis, 1999; Wilkinson, T. K., & Landis, 2005; Schmidt et al., 2007; Lundgren & Fergen, 2010). In a study on the ground beetle *Pterostichus melanarius*, the presence of a white clover cover crop increased the beetle's activity and predation and their spillover in the adjacent open crop (Chapman et al., 1999). Intraspecific diversity has received less attention concerning its influence on natural enemies (Koricheva & Hayes, 2018), but positive effects have been reported in spring cereals (Ninkovic et al., 2011; Grettenberger & Tooker, 2017) and soybean fields (Pan & Qin, 2014). For example, species richness of spiders and abundance of Linyphiidae spiders were increased by wheat cultivar mixtures related to a taller and more ramified vegetation layer (Chateil et al., 2013).

In our study, we were particularly interested in the application of those two diversification practices on wheat cropping systems. Firstly, wheat still represents one of the dominant crops worldwide and pests such as cereal aphids are responsible for considerable wheat crop losses (Dedryver et al., 2010; Shiferaw et al., 2013). Secondly, mixture of wheat cultivars (intraspecific diversification) receives a new surge of interest in the search for sustainable farming practices, because it offers higher yields, enhanced diseases regulation and a reduced impact of abiotic stressors (e.g. improved water use efficiency in water-limited environments) compared to monoculture (Mundt, 2002; Fang et al., 2014; Reiss & Drinkwater, 2018; Borg et al., 2018). Thirdly, intercropping cereals and legumes (interspecific diversification) has shown agronomical and environmental benefits such as nitrogen fixation, higher cereal protein content, weed control (Lithourgidis et al., 2011; Bedoussac et al., 2015; Verret et al., 2017; Vrignon-Brenas et al., 2018), but combining wheat with a legume-based cover crop for pest control has received little attention so far (Lopes et al., 2016).



Additionally, combining intra- and inter-specific diversity may result in an optimization of the mechanisms underlying biological pest control, as proposed by the ecostacking principle (i.e. combining several ecosystem service providers, which might be an organism, an interaction network, or even a habitat) (Gurr et al., 2017; Hokkanen, 2017). For example, we observed similar aphid infestation in a mixture of wheat cultivars compared with monoculture, but when intercropped with white clover, the mixture of wheat cultivars was less infested (Mansion-Vaquié *et al.*, *submitted* – see Chapter 3). Koricheva & Hayes (2018) suggest that genetic and species plant diversity may be more or less influential according to the arthropod trophic level (i.e. herbivore or natural enemies). How stacking genetic and species diversification practices and their resulting interactions may impact natural enemies and pest control have being largely overlooked so far and no investigation has been carried out on annual crops so far (Koricheva & Hayes, 2018).

Finally, even if diversification practices lead to an increase in the abundance of natural enemies, their presence is not a guarantee of their predation activity on the pest. Most of conservation biological control studies lack to relate the abundance of natural enemies to the assessment of their predation pressure in the fields (Furlong & Zalucki, 2010). Effect of habitat manipulation are often limited to the investigation of pest and natural enemies abundance (e.g. predator/prey ratio) and provide only a likelihood of biological control and not a proper quantitative measure of the impacts of the targeted pest and its natural enemies (Chisholm et al., 2014; Macfadyen et al., 2015). Intraguild predation, hyperparasitism or simply difficulties in prey location and/or access might lower the impact of natural enemies on herbivore populations (Letourneau et al., 2009). The use of sentinel preys, either real or artificial, may give a direct estimation of the predation pressure, especially in the case of comparative designs (Lövei & Ferrante, 2017).

In this paper we therefore aim to investigate the influence of both intraspecific (mixture of wheat cultivars) and interspecific (wheat-white clover association) diversification

practices on natural enemies and their predation pressure at the field scale in real farming conditions. We hypothesized that natural enemies' abundance and richness would be increased by each single diversification practice and by the double diversification scheme with a synergistic effect (i.e. greater than the summed effects resulting from each level). We also expected a lower effect from intraspecific compared to interspecific diversification practices, because the variation in traits is more limited at the genetic level compared to the species level (Cook-Patton et al., 2011; Barot et al., 2017). We estimated the pest regulation function of the diversification practices from the assessment of the parasitism rate of aphids and the potential predation pressure using two kinds of sentinel prey: aphid predation cards and dummy caterpillars. We finally also hypothesized that following an increased abundance of natural enemies; predation and parasitism rates would be higher for each single and combined diversification practices.

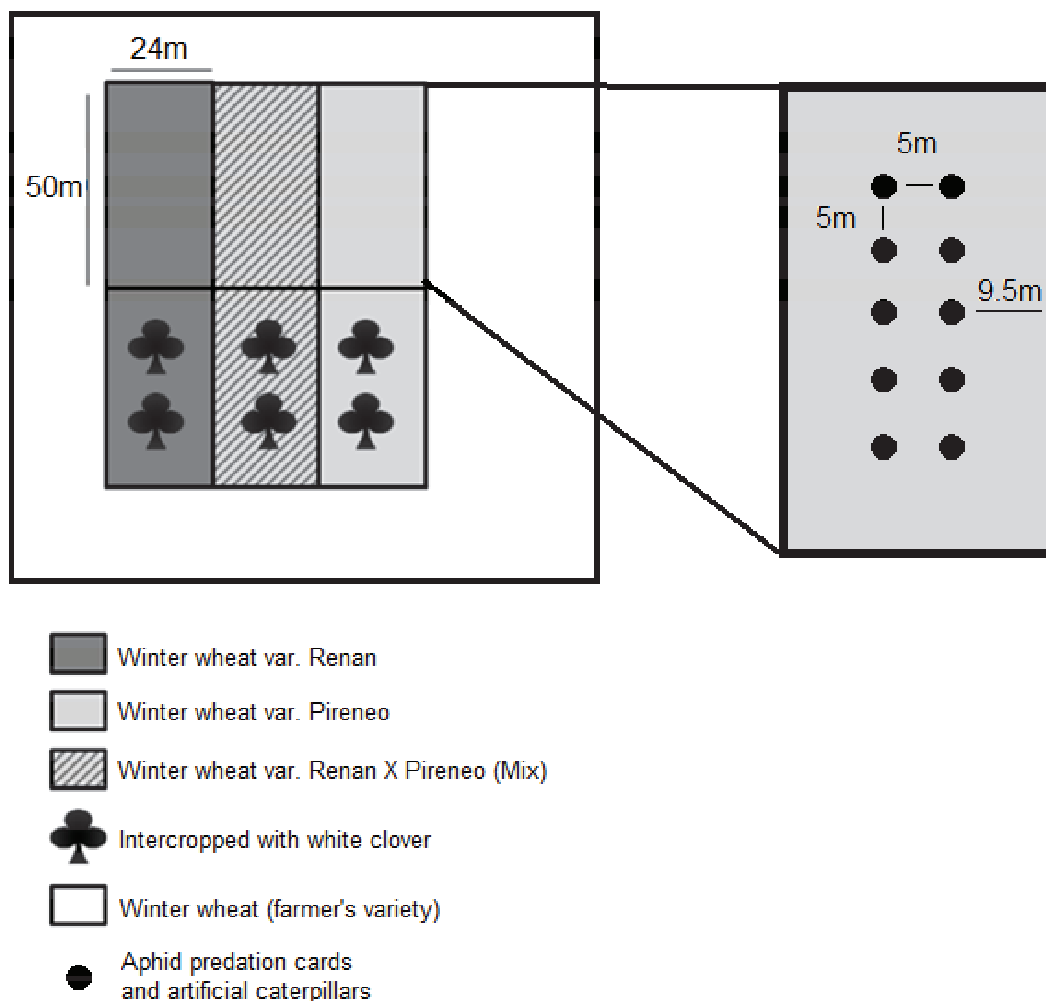
## **5.2 Material and methods**

### **5.2.1 Wheat and white clover cultivars**

For the intraspecific diversification practice, two different winter wheat (*Triticum aestivum* L.) cultivars were used in the experiment: Renan and Pireneo. The cultivar Renan is considered as the reference variety for French organic wheat farming (Dawson et al., 2013). The cultivar Pireneo is another common cultivar for French organic farming, characterized with 16 cm taller and covering less the ground than Renan (Fontaine et al., 2007). Mixture of wheat cultivars was composed by 50% Pireneo and 50% Renan, and blended in a concrete mixer to ensure homogeneity. For the white clover cover we used *Trifolium repens* var. Aberdai in 2016 and *Trifolium repens* var. Rivendel in 2017 (we used different seeds due to provisioning reasons in the different years). In 2017, white clover had difficulties to survive the winter.

### 5.2.2 Field experiment design

Twelve field experiments were established on organic fields in South-East of France (Auvergne-Rhône-Alpes region over two winter wheat cropping seasons (2015-2016 and 2016-2017). Among those fields only seven could be fully monitored and kept in the analyses. One field in 2016 and four fields in 2017 were excluded from the analysis because of different management by farmers and growth problems during crop development (e.g. strong weed infestation, no germination of clover seeds). The minimum distance between sites of the same growing season was 7 km. Mean field size was 5.2 ha ( $\pm$  4.9 S.D.). Each field experiment consisted of six treatments: 1) "Renan without clover"- sole stand of wheat monocultivar Renan, 2) "Pireneo without clover"- sole stand of wheat monocultivar Pireneo, 3) "Mix without clover"- sole stand of wheat mixture composed of both cultivars Renan and Pireneo, 4) "Renan with clover"- association of wheat monocultivar Renan with white clover, 5) "Pireneo with clover"- association of wheat monocultivar Pireneo with white clover, and 6) "Mix with clover"- association of the wheat mixture of Renan and Pireneo with white clover. Each treatment was applied on a 1200m<sup>2</sup> experimental plot (24 m X 50 m) established within an organic wheat field (Figure 5.1). The 2016 and 2017 fields were sown between October and November in 2015 and 2016, respectively. Winter wheat and white clover were sown simultaneously (with less than a 3-day interval in between) in an additive design at a seed density of 200 kg/ha and 5 kg/ha, respectively (as in Vrignon-Brenas et al., 2016a). In conformity with French organic farming regulation, no pesticides, herbicides, fungicides were used during the experiment. Following usual farmer practices, weeds were mechanically controlled (one or exceptionally two passages in February-March), except in the treatments with clover.



**Figure 5.1: Schematic representation of the field experimental design and the position of the aphid predation cards and the artificial caterpillars within each treatment.**

### 5.2.3 Predatory arthropods sampling

The composition of the arthropod natural enemy community was evaluated using two complementary sampling methods: pitfall traps for ground dwelling arthropods and visual counting for foliage dwelling arthropods. We only focused on predatory arthropods, and did not sample parasitoids.

Three pitfall traps were positioned in the central area of each treatment plot. The position of each pitfall trap was secured by positioning an outer gutter made of PVC ( $\text{Ø}$  10 cm, height 14 cm) in each hole. Each trap was composed by a plastic cup ( $\text{Ø}$  5 cm, volume 200 mL) filled with 100 mL propylene glycol 70% and a drop of odourless detergent. We used propylene glycol as a less toxic alternative to ethylene glycol (Thomas, 2008). For each

trap a funnel ( $\varnothing$  10 cm) was placed over the plastic cup and the outer gutter to increase the catch surface and avoid gaps between both cylinders. Traps were placed 10 m from each other and 15 m away from the plot edges. To reduce the bycatch, each trap was covered by a disposable plastic plate ( $\varnothing$  17 cm), supported by pegs. The traps were open for four periods of 48 h each, separated by 3 to 4 weeks between April and June 2016 and March and June 2017. A total of 561 samples were collected using this method. Collected samples were stored in 70 % ethanol.

Foliage dwelling arthropod predators were counted directly on wheat tillers every two weeks between March and June 2016 and between March and June 2017 (for a total of seven and six observations in 2016 and 2017, respectively). Each time, 30 wheat tillers were examined in each treatment along a central transect of 30 m, being one tiller every meter. Only taxa and growth stage with a potential predatory activity were recorded, i.e. adult and larvae of predatory lady beetles (Coccinellidae); larvae of hoverflies (Syrphidae); adult spiders (Araneae), rove and soldier beetles (Staphylinidae and Cantharidae, respectively), and also larvae of lacewings (Neuroptera). The latter were not found in our study.

Identification of predatory arthropods was to order (i.e., Araneae, Opiliones) or, in the case of beetles (Coleoptera), to family (i.e., Staphylinidae, Coccinellidae) or, in the case of ground beetles (Carabidae) to species using the identification keys of Jeannel (1941, 1942), Forel & Leplat (2003) and Coulon (2003, 2004a; b, 2005). For each ground beetle species identified, feeding habits (predator vs. other) and body size (more or less than 15 mm) were recorded based on information from Lindroth (1992), Ribera *et al.* (1999) and the public database <http://carabids.org> (Homburg *et al.*, 2013). Concerning spiders and opiliones, we counted only individuals at the adult stage (i.e. spider taller than 5 mm) (Reboulet, 1999).

#### 5.2.4 Predation pressure and parasitism

Predation pressure was quantified by assessing the predation rate on two kinds of sentinel prey: artificial caterpillars on the ground and aphid predation cards on wheat leaves. The artificial caterpillar is a 15 mm long and 3 mm thick cylinder, made of light green plasticine (Smeedi plus, V. nr. 776608, Denmark), using a modified garlic press as described by Howe et al. (2009). Each caterpillar was glued onto a small piece of reed or bamboo, to be handled without touching the plasticine. We placed 7 caterpillars, separated from each other by 5 m, in a line on both sides of the central width of each treatment (Figure 5.1; i.e. a total of 84 caterpillars per field per sampling event). At each location of a caterpillar, we placed a thin stake made of fiberglass. Artificial caterpillars were inspected in the field after 24 h for signs of predation attempts, using a hand-held magnifying glass (20×). If necessary, caterpillars were transported to the laboratory for detailed inspection and photographing. This method allows the identification of up to 14 different types of predators (Low et al., 2014; Lövei & Ferrante, 2017), but we only report the marks left by chewing insects in our study. We had in total 4 sampling events during May and June 2016 on 4 fields and 4 sampling events over March, April and June in 2017 on 2 fields.

Aphid predation cards (APC) were made of a piece of self-adhesive paper (2 cm<sup>2</sup>) on which was placed one live pea aphid *Acyrtosiphon pisum* (Harris) (4th instar nymphs and adults). To avoid natural enemies to stick on the APC, we dust a fine powder of dry wheat straw. APC were anchored to the wheat leave by a staple. We used pea aphids instead of cereal aphids such as *Sitobion avenae*, which is the main pest of wheat at spring, because they are larger and easier to manipulate. Therefore, only the predation rate of generalist predatory arthropods can be estimate by this method. We placed 7 APC on the wheat leaves in a line on both sides of the central width of each treatment, and separated from each other by 5 m (Figure 5.1: i.e. a total of 84 APC per field per sampling event). Each APC was precisely located nearby the stake used to locate artificial caterpillars. APC were observed after 24 h for

signs of predation attempts. As observed by Östman (2004), remnants of the abdomen or legs confirmed the predation events by foliage predators. We had 4 sampling events in 2016 during May and June.

Parasitism rate was calculated by dividing the number of mummies found on the plants by the number of live aphids plus mummies. Aphid and mummies (i.e. parasitized aphids) were counted directly on wheat tillers every two weeks between March and June 2016 and between March and June 2017 (for a total of seven observations each year). At each sampling event, 30 wheat tillers were examined in each treatment along a central transect of 30 m, being one tiller every meter. Densities of aphid and mummies were pooled over 30 wheat tillers for each sampling. The results concerning aphid occurrence and infestation are presented in Chapter 3.

#### 5.2.5 Statistical analyses

For each sampling method (pitfall traps or visual counting), taxonomic orders representing < 10 % of the total catch were excluded from the statistical analyses. The activity-density of the remaining ground dwelling predators, the species richness of ground beetles, and the abundance of the remaining foliage dwelling predators were modelled with Generalized Linear Mixed Models (GLMMs) using the *glmer* function *lme4* package (Bates et al., 2015) with negative binomial error distribution and we set manually the dispersion parameter  $\theta$  to account for overdispersion. The predation rate on artificial caterpillars and APC, were modelled with GLMMs with binomial error distribution, and the parasitism rate with a Gaussian distribution using the *lmer* function. Missing caterpillars and APC were considered lost and were excluded from the analyses.

Five (G)LMs were fitted on each measured variables with the following fixed covariates: *Wheat* treatment (categorical with three levels: Renan, Pireneo and Mix; default level is set as Renan, because it is the reference wheat cultivar in French organic agriculture)

and *Clover* treatment (categorical with two levels: with and without). The following random intercepts were used for each model: *Sampling date*, *Experimental plot* and *Field* to account for variation in treatments due to site effects. Year was not included, because it was found poorly influential in preliminary analyses. Model 1 considered all the interactions among fixed effects; Model 2 considered the additive effect of both fixed-effect variables; Model 3 considered only *Wheat* treatment; Model 4 considered only *Clover*; Model 5 was the null model. The best model for each measured variable was selected as that with the lowest Akaike information criterion with a second order correction (AICc) adapted for small samples (Burnham & Anderson, 2002). The significance of fixed effects from the selected model and their interaction was determined with an F-test with a Kenward-Roger correction for LMMs or likelihood ratio test (LRT) for GLMMs as implemented in the *mixed* function in the *afex* package (Singmann et al., 2018). The Kenward-Roger correction is used to calculate the denominator degrees of freedom, that we round up to the nearest unit.

Additionally, we tested the relationship between predation rate on artificial caterpillars by chewing insects and the activity density of spiders and carabids with LMMs, with the following random structure: *Sampling date* as random intercept, and *Field* as random slope to account for variation in treatments due to site effects. We also tested this relationship with only ground beetles  $\geq 15$  mm in length, because we may assume that carabids  $< 15$ mm would not attack prey larger than themselves.

Permutational analysis of variance (PERMANOVA) was used to analyze how diversification practices affect the species composition of predatory ground beetle communities. PERMANOVA was realized using the *vegan* package in R (function *adonis*) (Oksanen et al., 2013) and with the following fixed covariates: *Wheat* treatment, *Clover* treatment and *Site*. PERMANOVA results were calculated based on 50 000 permutations. The effect of diversification practices and site on species composition is illustrated using a



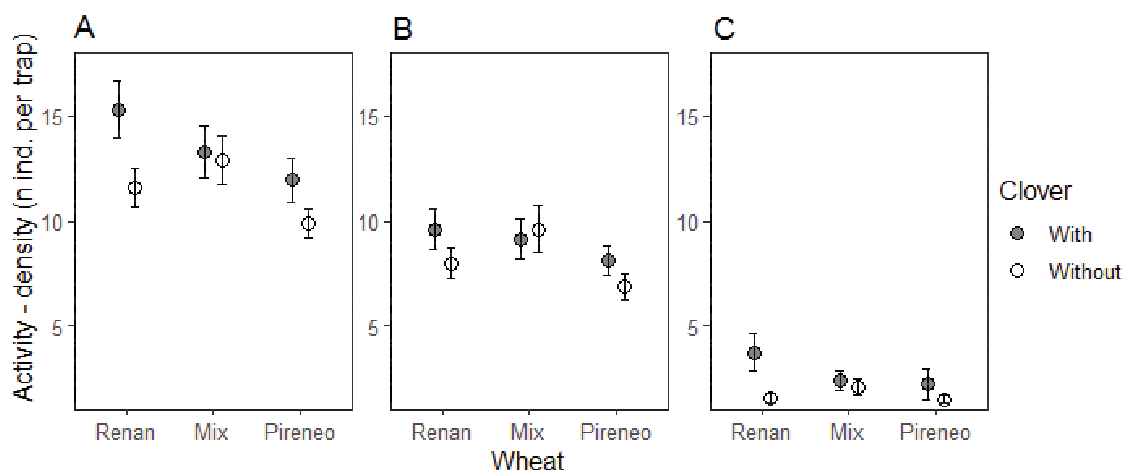
correspondence analysis. All statistical analyses were conducted using R, version 3.4.3 (R Development Core Team, 2017).

## **5.3 Results**

### **5.3.1 Ground dwelling predatory arthropods**

A total of 4234 spiders and 1106 predatory ground beetles were collected by pitfall trapping (Table 5.1). We identified 35 species of predatory ground beetles, among which *Anchomenus dorsalis*, *Brachinus sclopeta*, *Trechus quadristriatus* and *Pterostichus melanarius* represented almost 80% of the total catch (for details see Table 5.2). Each other taxa of predatory arthropods captured by pitfall traps represented less than 10% of the total (Table 5.1).

When all predatory arthropods were pooled together (i.e., Araneae, Carabidae, Staphylinidae, Coccinellidae, Opiliones), the activity-density was significantly higher in the treatments with clover, as well as in stands Renan (with and without clover) compared to Pireneo monocultivar (Table 5.3, Figure 5.2.A). The activity-density of spiders tended to be higher in the treatments with clover, but we found no significant effect of the diversification treatments (Table 5.3; Figure 5.2.B). The activity-density of the predatory ground beetles, was significantly higher in, and varied with the wheat treatments but the effect was not significant (Table 5.3; Figure 5.2.C).



**Figure 5.2:** Activity-density (mean number of individuals per trap  $\pm$  S.E.) of (A) all ground dwelling predatory arthropods, (B) spiders and (C) predatory ground beetles according to wheat and clover treatments (n = 561). All sampling dates are included.

**Table 5.1: Mean activity-density (no. of individuals per trap  $\pm$  S.E.) of ground dwelling predatory arthropods collected on a total of 561 pitfall traps, and mean number of foliage dwelling predatory arthropods (no. of individuals per 30 wheat tillers  $\pm$  S.E.) counted visually on 8460 wheat tillers in the different wheat and clover treatments over the sampling seasons in 7 organic wheat fields in south-eastern France.**

Vegetation layer monitored	Taxonomic group	Without clover			With clover			TOTAL cumulated	Percentage %
		Renan	Mix	Pireneo	Renan	Mix	Pireneo		
Ground dwelling (pitfall traps)	Araneae	8.0 $\pm$ 0.7	9.6 $\pm$ 1.1	6.9 $\pm$ 0.6	9.6 $\pm$ 0.9	9.1 $\pm$ 1.0	8.1 $\pm$ 0.7	4234	<b>68.6</b>
	Coleoptera:								
	<i>Carabidae</i>	1.6 $\pm$ 0.2	2.1 $\pm$ 0.4	1.5 $\pm$ 0.2	3.7 $\pm$ 0.9	2.4 $\pm$ 0.5	2.2 $\pm$ 0.8	1106	<b>17.9</b>
	<i>Staphylinidae</i>	1.4 $\pm$ 0.5	0.8 $\pm$ 0.2	1.1 $\pm$ 0.3	1.2 $\pm$ 0.2	1.4 $\pm$ 0.3	1.1 $\pm$ 0.2	568	<b>9.2</b>
	<i>Coccinellidae</i>	0.1 $\pm$ 0.04	0.1 $\pm$ 0.05	0.1 $\pm$ 0.02	0.1 $\pm$ 0.06	0.1 $\pm$ 0.04	0.1 $\pm$ 0.05	56	<b>0.9</b>
	Opiliones	0.6 $\pm$ 0.2	0.3 $\pm$ 0.0	0.4 $\pm$ 0.1	0.6 $\pm$ 0.2	0.2 $\pm$ 0.06	0.4 $\pm$ 0.1	210	<b>3.4</b>
	<b>TOTAL cumulated</b>	<b>963</b>	<b>1083</b>	<b>831</b>	<b>1241</b>	<b>1052</b>	<b>1004</b>	<b>6174</b>	<b>100</b>
Foliage dwelling (visual transect)	Araneae	0.7 $\pm$ 0.2	0.6 $\pm$ 0.2	0.7 $\pm$ 0.2	0.9 $\pm$ 0.3	0.8 $\pm$ 0.2	1.0 $\pm$ 0.3	221	<b>54.0</b>
	Coleoptera:								
	<i>Coccinellidae</i>	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.3 $\pm$ 0.08	0.3 $\pm$ 0.1	0.2 $\pm$ 0.07	0.3 $\pm$ 0.1	89	<b>21.8</b>
	<i>Cantharidae</i>	0.1 $\pm$ 0.04	0.3 $\pm$ 0.2	0.1 $\pm$ 0.07	0.2 $\pm$ 0.1	0.2 $\pm$ 0.08	0.1 $\pm$ 0.06	43	<b>10.5</b>
	<i>Staphylinidae</i>	0.04 $\pm$ 0.03	0.04 $\pm$ 0.03	0.1 $\pm$ 0.07	0.04 $\pm$ 0.03	0.1 $\pm$ 0.07	0.02 $\pm$ 0.02	17	<b>4.2</b>
	Diptera:								
	<i>Syrphidae</i> (larvae)	0.2 $\pm$ 0.08	0.2 $\pm$ 0.1	0.2 $\pm$ 0.09	0.1 $\pm$ 0.06	0.1 $\pm$ 0.05	0	39	<b>9.5</b>
	<b>TOTAL cumulated</b>	<b>64</b>	<b>77</b>	<b>70</b>	<b>71</b>	<b>64</b>	<b>63</b>	<b>409</b>	<b>100</b>

**Table 5.2: Predatory carabid species collected in pitfall traps in organic wheat fields in south-eastern France: total numbers, percentage and accumulated percentage (% Acc.) of the dominant species (i.e. representing  $\geq 1\%$  of the total).**

<b>Species</b>	<b>Total</b>	<b>%</b>	<b>% Acc.</b>
<i>Anchomenus dorsalis</i>	444	40%	40%
<i>Brachinus sclopeta</i>	177	16%	56%
<i>Trechus quadristriatus</i>	161	15%	71%
<i>Pterostichus melanarius</i>	88	8%	79%
<i>Carabus auratus</i>	54	5%	84%
<i>Bembidion properans</i>	28	3%	86%
<i>Calathus fuscipes</i>	16	1%	88%
<i>Brachinus explodens</i>	13	1%	89%
<i>Carabus monilis</i>	12	1%	90%
<i>Cylindera germanica</i>	12	1%	91%
<i>Agonum muelleri</i>	12	1%	92%
<i>Bembidion lampros</i>	11	1%	93%
<i>Nebria salina</i>	11	1%	94%
<i>Brachinus elegans</i>	8	1%	95%
<i>Microlestes maurus</i>	7	1%	95%
<i>Notiophilus substriatus</i>	7	1%	96%
Total species (35)	1106	100%	100%

**Table 5.3: Results of the general linear mixed models GLMMs selection relating wheat treatments (W) and clover treatments (C) to response variables concerning predatory arthropods. Significance of their fixed effects and their interaction was determined with F-test with a likelihood ratio test. Best model for each response variable was selected with the lowest AICc value and are underlined. Only the best two models are presented for each response variable.  $\Delta$ AICc represents the difference in AICc with the second closest model.  $\Delta$ AICc-Null represents the difference in AICc with the null model. Model 1 = W\*C; Model 2 = W+C; Model 3 = W; Model 4 = C; Model 5 is the null model. For each model, the variable Sampling date, Experimental plot and Field was included as a random effect.  $\Theta$  is the dispersion parameter for models with a negative binomial error distribution. Significant p-values ( $\leq 0.05$ ) are shown in bold.**

	Response variable	Model	$\theta$	Wheat (W)	Clover (C)	W:C	df	AICc	$\Delta$ AICc	$\Delta$ AICc - Null	
Ground dwelling predatory arthropods	All predators activity-density	<u>Model 2</u>	4	$\chi_{(2,6)} = 8.20$ <b>p = 0.02</b>	$\chi_{(1,7)} = 7.21$ <b>p &lt; 0.01</b>	-	8	3198.7	2.4	7.8	
		Model 1		$\chi_{(2,8)} = 8.60$ <b>p = 0.01</b>	$\chi_{(1,9)} = 7.59$ <b>p &lt; 0.01</b>	$\chi_{(2,8)} = 1.77$ p = 0.41	10	3201.1		5.4	
	Spider activity-density	<u>Model 2</u>	3.2	$\chi_{(2,6)} = 5.14$ p = 0.08	$\chi_{(1,7)} = 3.44$ p = 0.06	-	8	2910.2	0.9	1.9	
		Model 4		-	$\chi_{(1,5)} = 2.90$ p = 0.09	-	6	2911.1		1.0	
	Ground beetle activity-density	<u>Model 1</u>	1.9	$\chi_{(2,8)} = 5.71$ p = 0.06	$\chi_{(1,9)} = 3.79$ <b>p = 0.05</b>	$\chi_{(2,8)} = 4.87$ p = 0.09	10	1687.1	0.7	2.7	
		Model 2		$\chi_{(2,6)} = 5.26$ p = 0.07	$\chi_{(1,7)} = 3.34$ p = 0.07	-	8	1687.8		2.0	
	Ground beetle species richness	<u>Model 1</u>	NA	$\chi_{(2,7)} = 4.57$ p = 0.10	$\chi_{(1,8)} = 2.47$ p = 0.11	$\chi_{(2,7)} = 5.20$ p = 0.07	9	1223.1	1.1	1.1	
		Model 5		-	-	-	5	1224.2		0	
	Foliage dwelling predatory arthropods	All predators	<u>Model 5</u>	2	-	-	-	5	899.5	1.6	0
			Model 4		-	$\chi_{(1,6)} = 0.51$ p = 0.47	-	6	901.1		1.6
Spider abundance		<u>Model 5</u>	NA	-	-	-	4	629.5	0.5	0	
		Model 4		-	$\chi_{(1,4)} = 1.49$ p = 0.22	-	5	630.0		0.5	
Lady beetle abundance		<u>Model 4</u>	NA	-	$\chi_{(1,4)} = 2.96$ p = 0.09	-	5	413.6	0.9	0.9	
		Model 5		-	-	-	4	414.5		0	
Soldier beetle abundance		<u>Model 5</u>	NA	-	-	-	4	197.2	1.2	0	
		Model 3		$\chi_{(2,4)} = 2.99$ p = 0.23	-	-	6	198.4		1.2	

The species richness of predatory ground beetles was in average 1.1 ( $\pm$  0.06 S.E.), and we observed no significant effects of diversification. Our results revealed that the species composition of predatory ground beetle communities were significantly related to sites and indicated changes in composition between clover treatments depending on the site (significant clover treatment : site interaction; Table 5.4; Figure 5.3). We also observed a slightly significant effect of wheat treatments on the species composition of predatory ground beetle communities.

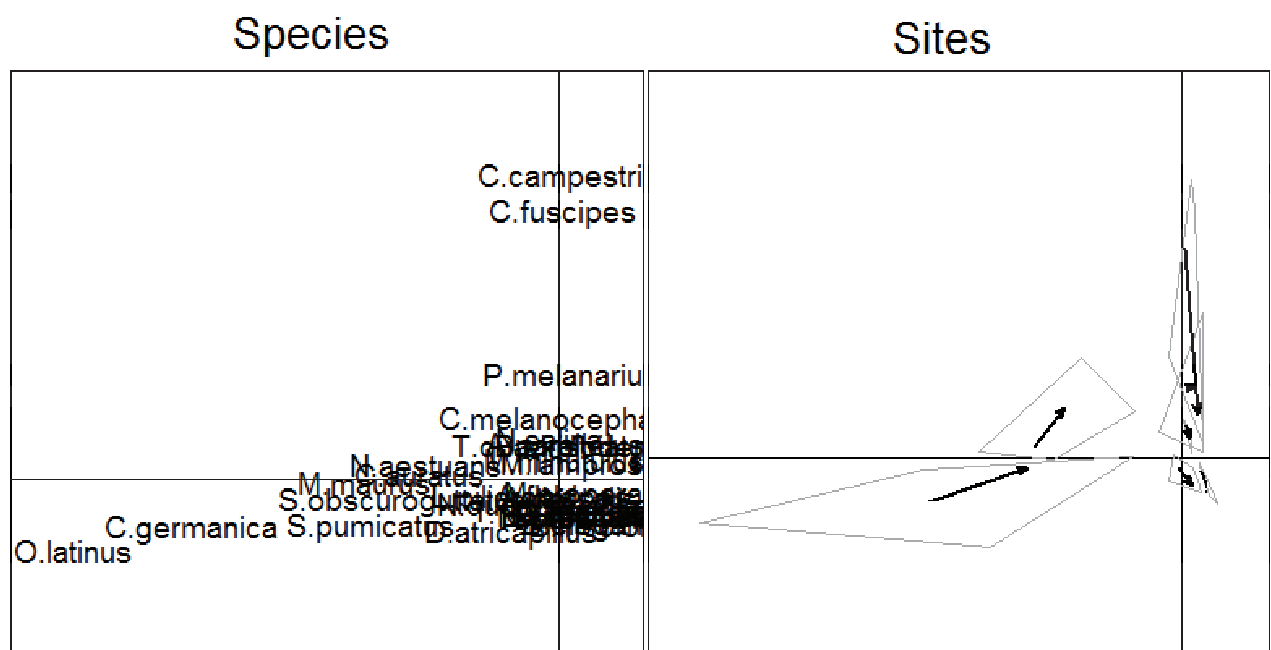


Figure 5.3: Correspondence analysis plots of predatory ground beetle communities (Species) sampled in 7 different fields (Sites). Variance explained by axis: CA1, 16.2%; CA 2, 14.0%. Arrows represent the shift in species composition from plots without towards with clover cover. The grey polygons represent the variation in species composition within each field.

Table 5.4: Effects of diversification (wheat and clover treatments) and sites on predatory ground beetle community composition based on permutational analysis of covariance. Significant effects are shown in bold (P-value  $\leq$  0.05).

Variables	Predatory ground beetle			
	df	F	R <sup>2</sup>	p-value
Clover	1	1.81	0.02	0.08
<b>Wheat</b>	2	1.75	0.04	<b>0.05</b>
<b>Site</b>	6	9.44	0.58	<b>&lt;0.001</b>
Clover : Wheat	2	0.84	0.02	0.64
<b>Clover : Site</b>	6	1.53	0.09	<b>0.04</b>
Wheat : Site	12	1.06	0.13	0.38
Residuals	12		0.12	
Total	41		1.00	

### 5.3.2 Foliage dwelling predatory arthropods

In total, we observed 409 individual predatory arthropods on a total of 8460 wheat tillers. Most abundant taxa were Araneae (54.0 %), Coccinellidae, including adults and larvae, (21.8 %) and Cantharidae (10.5 %). Each other taxa of predatory arthropods observed (e.g. Staphylinidae and larvae of Syrphidae) represented less than 10 % of the total (Table 5.1).

When all foliage dwelling predatory arthropods were pooled together, we found no evidence of a global effect of the diversification treatments on the abundance of foliage dwelling arthropod predators (Table 5.3, Figure 5.4.A). We observed the same results concerning the foliage dwelling spiders and soldier beetles (Table 5.3, Figure 5.4.B & 5.4.D). Lady beetles (adults and larvae) were less abundant on wheat leaves when intercropped with clover (0.26 ind.  $\pm$  0.06 S.E.) compared to wheat cultivated in monoculture (0.38 ind.  $\pm$  0.07 S.E.) but this difference was not significant (Table 5.3, Figure 5.4.C).

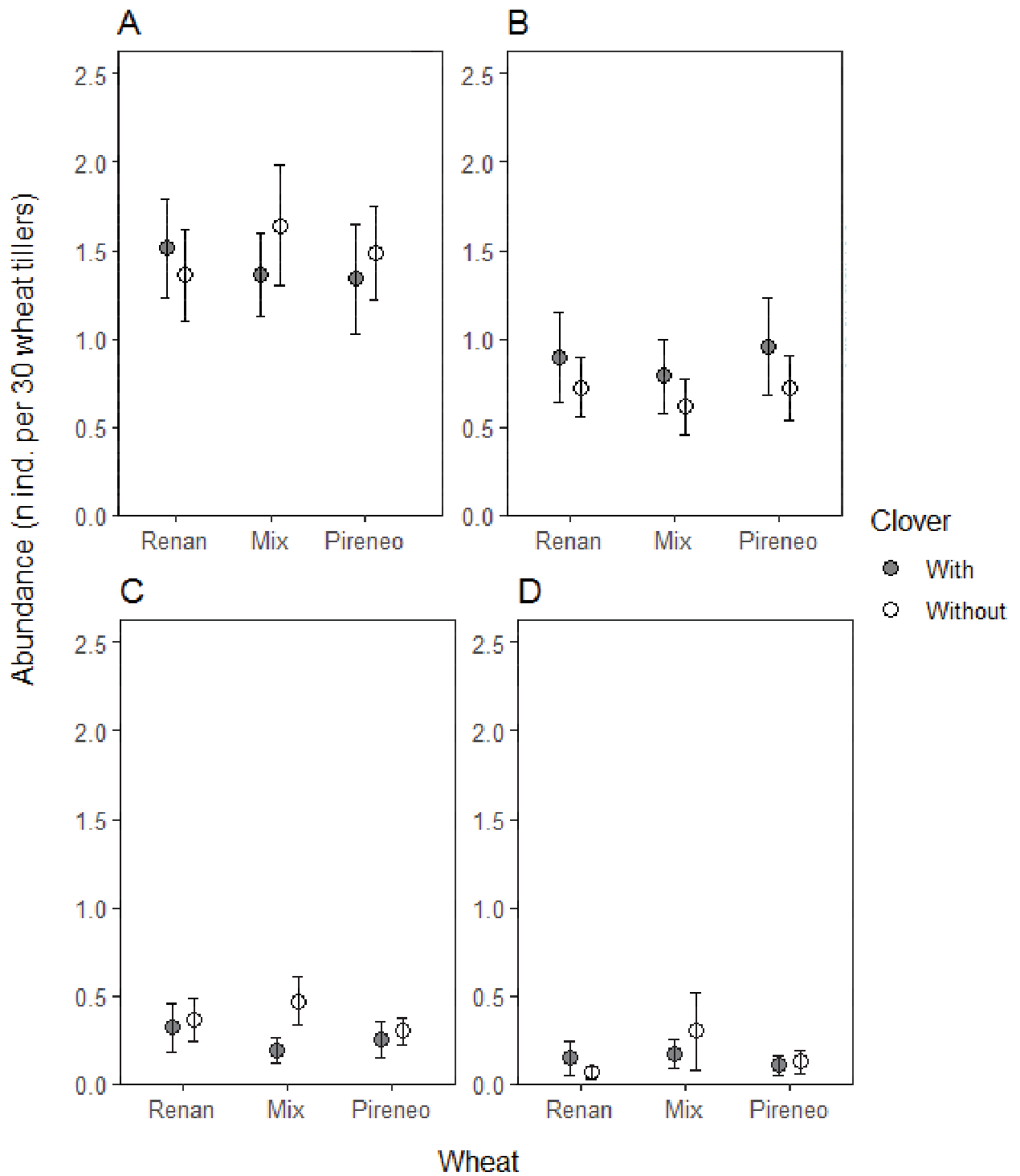
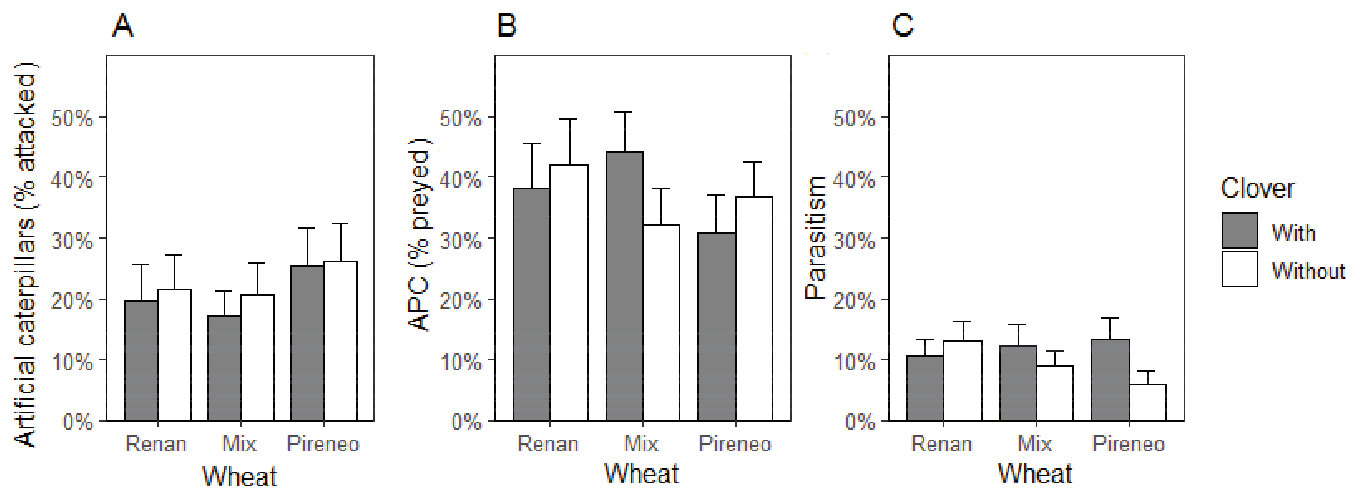


Figure 5.4: Abundance (mean number of individuals per 30 wheat tillers  $\pm$  S.E.) of (A) all foliage dwelling predatory arthropods, (B) spiders, (C) lady beetles and (D) soldier beetles according to wheat and clover treatments ( $n = 282$ ). All sampling dates are included.

### 5.3.3 Predation pressure and parasitism

At the ground level, 22 % (n = 363/1681) of the artificial sentinel preys were attacked by chewing insects after 24 h of exposure. Less than 1 % of caterpillars were lost. We found no significant effect of the diversification treatments on the predation rate on artificial caterpillars by insects (Table 5.5, Figure 5.5.A).



**Figure 5.5: Predation pressure assessment: (A) artificial caterpillars (mean % attacked after 24h  $\pm$  S.E.; n=1665); (B) aphid predation card (mean % preyed after 24h  $\pm$  S.E.; n= 1242); (C) parasitism rate (mean %  $\pm$  S.E.; n=282); according to wheat and clover treatments.**

Predation rate did not increase with the abundance of spiders (Figure 5.6.A; F-test on LMM:  $F_{1,3} = 0.16$ , p-value = 0.72), not with the abundance of predatory ground beetles (Figure 5.6.B; F-test on LMM:  $F_{1,3} = 0.42$ , p-value = 0.57). We did not find a significant relationship between the activity-density of large ( $\geq 15$  mm) predatory ground beetles and the predation rate on artificial caterpillars (Figure 5.C; F-test on LMM:  $F_{1,4} = 0.47$ , p-value = 0.53). Those relationships varied among fields (Figure 5.6).



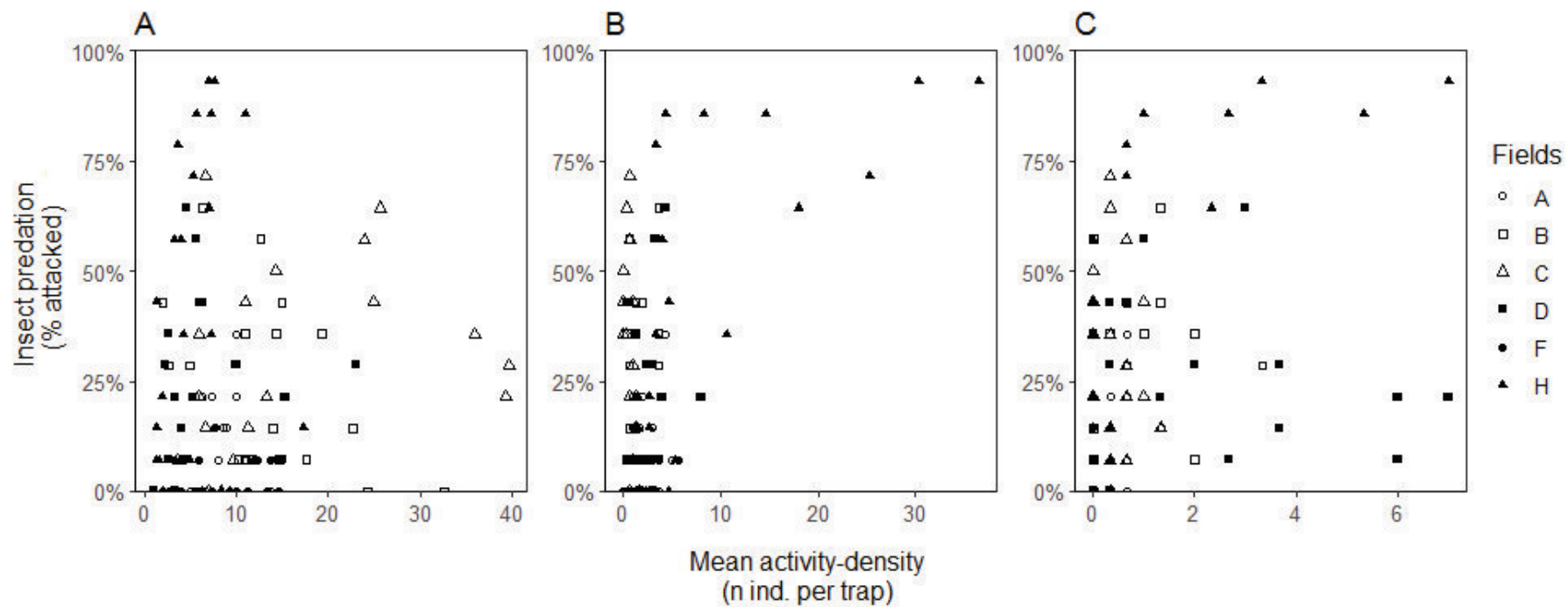


Figure 5.6: Relationship between predation on artificial caterpillars (mean % attacked after 24 h) and abundance of (A) spiders; (B) predatory ground beetles and (C) large ( $\geq 15$  mm) predatory ground beetles (mean number of individuals per trap and per sampling occasion) according to the fields ( $n=120^*$ ). \*Only sampling dates, for which both measures were realized in parallel, were analysed.

At the foliage level, 35% (n = 460/1249) of the APC were preyed upon after 24 h of exposure. Only 0.56 % APC were lost. We found no evidence of a global effect of the diversification treatments on the predation rate of the APC (Table 5.5, Figure 5.5.B).

Parasitism rate was in average below 11 % (Figure 5.5.C), with in average 14 % for the year 2016 and 2 % for the year 2017. We found no significant effect of the diversification treatments on parasitism in overall (Table 5.5).

**Table 5.5: Results of the (general) linear mixed models (G)LMMs selection relating wheat treatments (W) and clover treatments (C) to response variables related to predation pressure and parasitism. Significance of their fixed effects and their interaction was determined with F-test with a Kenward-Roger correction for LMMs or likelihood ratio test for GLMMs. Best model for each response variable was selected with the lowest AICc value and are underlined. Only the best two models are presented for each response variable.  $\Delta$ AICc represents the difference in AICc with the second closest model.  $\Delta$ AICc-Null represents the difference in AICc with the null model. Model 1= W\*C, Model 2= W+C, Model 3= W, Model 4= C;, Model 5 is the null model. For each model, the variable Sampling date, Experimental plot and Field was included as a random effect.**

Response variable	Model	Wheat (W)	Clover (C)	W:C	df	AICc	$\Delta$ AICc	$\Delta$ AICc - Null
Predation rate on aphid predation cards	<u>Model 5</u>	-	-	-	4	1460.2	1.4	0
	Model 4	-	$\chi_{(1,4)} = 0.64$ $p = 0.43$	-	5	1462.6		1.4
Predation rate on artificial caterpillars	<u>Model 5</u>	-	-	-	4	1420.5	0.5	0
	Model 3	$\chi_{(2,4)} = 3.57$ $p = 0.17$	-	-	6	1421.0		0.5
Parasitism rate	<u>Model 5</u>	-	-	-	5	126.7	6.3	0
	Model 4	-	$\chi_{(1,5)} = 1.61$ $p = 0.21$	-	6	120.4		6.3

## **5.4 Discussion**

The present study aimed at verifying the hypotheses that increasing intraspecific (mixture of wheat cultivars) and interspecific (wheat-white clover association) plant diversity, as well as the combination of both, enhance natural enemies and their potential of pest regulation at the field scale. We observed different influence of the diversification practices according to the vegetation layer occupied by different taxa of natural enemies. At the ground level, intercropping wheat with clover tended to benefit predatory arthropods as a whole, and we observed a preference for one wheat cultivar, but not for the mixture of cultivar. At the foliage level, single diversification practices did not influence natural enemies in overall, except a slight negative impact of the intercropping on lady beetles. Combining wheat cultivar mixture and wheat-white clover intercropping on the same cultivated area did not outperform each practice individually in attracting natural enemies. The most diversified treatment (i.e. wheat cultivar mixture intercropped with clover) was indeed not the richest one in term of predators' abundance. Potential of predation (whatever the sentinel prey used) and parasitism were not influenced by any of the diversification practices in our study.

### **5.4.1 Natural enemies**

Our observations support partially the hypotheses that natural enemies' abundance would be increased by each single diversification practice. In our study, intercropping wheat with a cover of white clover increased the overall abundance of ground dwelling natural enemies, especially the predatory ground beetles. These results are consistent with other studies investigating the impacts of cover crops on ground dwelling arthropods (Carmona & Landis, 1999; Prasifka et al., 2006; Holland et al., 2016). The species richness of ground beetles may seem low in our study ( $1.1 \pm 0.06$  S.E.), but the pitfall traps were only open for a 48 h period. It is difficult to compare absolute values with other studies, because there is a large variability in the sampling design of pitfall traps (Brown & Matthews, 2016). We observed that the addition of a clover cover influenced the assemblages of predatory ground beetle species,

meaning that the influence of intercropping is not uniform across arthropod species. Different response to the presence of a cover crop according to the ground beetle species was already reported in other cropping systems such as cabbage intercropped with white clover (Booij et al., 1997; Chapman et al., 1999). Moreover, we observed that the influence of intercropping on ground beetle communities was site-dependent. Several factors, such as crop rotation and other farming practices (e.g. tillage) or landscape composition, contribute towards forming field-specific carabid assemblages, and may interfere with the effect of interspecific diversification (Tonhasca, 1993; Kromp, 1999; Holland & Luff, 2000; Purtauf et al., 2005). Concerning ground dwelling spiders, other studies reported an increase in abundance, especially Linyphiidae spiders, in the presence of clover cover (Gravesen, 2008), while it remained only a tendency in our study. This is surprising because Sunderland & Samu (2000) observed that interspersed diversification (i.e. the companion plant is mixed with the target crop, contrary to row intercropping) increased spider abundance in 80% of the cases. Among the possible explanation, intraguild predation between ground dwelling beetles and spiders may interfere with the influence of interspecific diversification on spiders (Lang, 2003). Concerning foliage dwelling arthropods, intercropping wheat with white clover had not significant effect, contrarily to other type of wheat-based intercrops such as oilseed rape or mung bean (Wang et al., 2009; Xie et al., 2012). White clover did not provide floral resources in our experiment and may be less attractive to foliage dwelling arthropods compared to flowering crop such as oilseed rape or mung bean.

Contrary to our hypotheses, mixing wheat cultivars did not influence abundance of natural enemies, even if we expected a lower effect from intraspecific compared to interspecific diversification practices (Cook-Patton et al., 2011; Barot et al., 2017). There are only few studies investigating the influence of cultivar mixtures on natural enemies under field conditions. The influence of intraspecific diversity may not be uniform across arthropod species, similarly to the results we found on intercropping. We observed for example that

intraspecific diversification slightly impacted the composition of the assemblages of predatory ground beetle species. Moreover, among other ground dwelling predatory taxa, only Linyphiidae spiders responded positively to increased number of wheat cultivars but not predatory ground beetles (Chateil et al., 2013; Dubs et al., 2018). Variance in plant height in cultivar mixtures was suggested to enrich microclimate habitats for natural enemies (Barot et al., 2017), but on the fields this hypothesis is neither verified by our study (see also Mansion-Vaquié *et al.*, *submitted* – see Chapter 3) nor by Dubs *et al.* (2018). We observed a slight difference between the two wheat cultivars in the abundance of ground dwelling predatory arthropods, with Renan being more attractive than Pireneo and the mixture of both cultivars being mostly in between the two. This difference may be related to the lower abilities of Pireneo to cover the ground compared to Renan (Fontaine et al., 2007), and that we also observed in the fields in a previous study (Mansion-Vaquié *et al.*, *submitted* – see Chapter 3). Interestingly, we could report a reduced abundance of aphids in Renan compared to Pireneo (Mansion-Vaquié *et al.*, *submitted* – see Chapter 3), which may indicate a potential top down control from the natural enemies. However, this was not confirmed by our measures of predation rate through the use of surrogate prey, as we did not record any influence of any diversification practice.

#### 5.4.2 Biocontrol potential

Our observations do not support the hypothesis that predation and parasitism rates would be higher for each single and combined diversification practices. At the ground level, predation on artificial caterpillars was not influenced by any diversification practices, despite an increased abundance in ground dwelling natural enemies in intercropped treatments. It highlights the importance to include measures of pest suppression or predation rate when investigating management practices for improving pest control (Furlong & Zalucki, 2010). Generalist predators, such as ground dwelling beetles and spiders, are characterized by their opportunistic feeding habits (Symondson et al., 2002) and two types of polyphagy (i.e.

intraguild predation and feeding on alternative prey) could limit the effectiveness of conservation biological control (Lang, 2003; Prasad & Snyder, 2006). The presence of a cover crop may have favored alternative preys (e.g. springtails) to the detriment of artificial sentinel preys, as for spiders that have been shown to eventually switch from targeted aphid preys to springtails (Gravesen, 2008; Kuusk & Ekbom, 2010). It may also be argued that artificial caterpillars, as used in our study, are not appealing enough to predators compared for example to real sentinel prey, but some studies temper such assumption. Ferrante *et al.* (2017b) demonstrated that the predatory carabid *Pterostichus melanarius* (Illiger) did not show preference for unwounded alive caterpillars compared to artificial odourless caterpillars made of plasticine, advocating that this method is valuable to estimate the predation pressure in the field, especially in the case of comparative designs (Lövei & Ferrante, 2017). Moreover, if the size of the artificial caterpillars may discourage small predators (Lövei & Ferrante, 2017), we did not observe any relationship between the activity - density of large ( $\geq 15$  mm) ground beetles and the attack rate on artificial caterpillars, contrary to Mansion-Vaquié *et al.* (2017). The comparison may however be biased due to the fact that they investigated the relationship between attack rates after 24h of caterpillars' exposure and the mean number of large ground beetles captured over 7 days, while we analyzed this relationship based on a capture duration of 2 days. It is therefore likely that, we underestimate the activity-density of large ground beetles compared to them. An investigation of other traits of natural enemy community such as body length, or habitat preferences should be deepened to better understand the shifts in community composition that might result from interspecific diversification and the resulting impacts on predation rates (Rusch *et al.*, 2015).

At the foliage level, removal rate on aphid predation cards was not influenced by any diversification practices, which may be related to the absence of effects on foliage dwelling natural enemies. Aphid removal rate on pea aphid predation cards was indeed found to correlate with predator abundance (Östman, 2004; Ximenez-Embun *et al.*, 2014). We have

only measured predation activity of generalist predators, because we used a sentinel prey (*Acyrtosiphon pisum*) which is not the aphid pest naturally occurring on wheat. However, generalist predators have been observed foraging on aphid predation cards in fields of spring barley or alfalfa (Östman, 2004; Ximenez-Embun et al., 2014). Parasitism rates were not influenced by any diversification practices in our study. Variable results have been reported concerning the influence of interspecific diversification on parasitism in wheat or cruciferous crops (Hooks & Johnson, 2003; Lopes et al., 2016). The non-host plant species identity may be responsible for such variations. For example, intercrops including appropriate floral resources may be more successful to attract parasitic wasps within the field, as some species exploit pollen or extra-floral nectar (Wäckers et al., 2008). It was also observed that the impact of intercropping on the foraging behavior of parasitoids depends on the parasitoids species and their host range (Perfecto & Vet, 2003). Increasing intraspecific diversity was found to neither impact parasitoid abundance and nor parasitism rate, although the authors did not specified if the system studied concerned crops or wild plant species (Koricheva & Hayes, 2018). Olfactory tests showed that combination of two barley cultivars were generally not attractive to parasitoids (Glinwood et al., 2009). Additionally, the effect of intraspecific diversification on pest regulation is known to be influenced by the level of biotic pressure (Power, 1991). The level of aphid parasitism was low in our study, if we refer to Sigsgaard (2002) and Holland et al. (2008) also measuring parasitism rate in wheat fields, and well below the rate of 32–36% under which successful classical biocontrol has never been reported (Hawkins & Cornell, 1994). Such low level of parasitism may be among the plausible explanations for the absence of an effect of diversification. Moreover, based on the disruptive crop hypothesis (Vandermeer, 1989; Poveda et al., 2008), it is expected that aphids have difficulties to locate and colonize their host plants within diversified cropping systems. Finally, it was suggested that as aphid parasitoids are restricted to aphid prey (Powell & Pell, 2007), increasing vegetation diversity may therefore also reduce their own foraging efficiency (Gols et al., 2005).

## **5.5 Conclusion**

To conclude, our results suggest that intercropping wheat with a cover crop may be attractive for ground dwelling arthropods, but without resulting into increased pest control. Wheat variety mixtures are not likely to benefit predatory arthropods in overall. The combination of both diversification practices did not result in any emergent properties concerning an improved biological control, under organic farming conditions. Our study emphasizes the importance of measuring pest suppression or predation rate when investigating management practices for pest control.

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## **Chapter 6:**

### Discussion

## **6.1 Overview of the main results and hypotheses validation**

There is a renewed interest for diversification practices in cropping systems, because they may optimize the delivery of multiple ecosystem services in agroecosystems. Increasing intrafield plant diversity has been shown to regulate pest populations in various agroecosystems. Cultivar mixtures of a crop species (intraspecific diversity) or associations of a crop and a companion plant (interspecific diversity) are both considered as promising agroecological practices for low-input or organic agriculture systems by providing several ecosystem services in addition to the production one, such as nitrogen fertilization and pest, disease and/or weed control . The novelty of the work presented here was to assess if the combination of both intra- and interspecific diversification practices in annual cropping systems and under real farming conditions on farmers' fields would result in improved pest control, while not constraining the agronomic performance of the crop.

Based on our field experiments, the most diversified treatment that combines intra- and interspecific diversity, did not outperform each practice individually in reducing aphid populations, attracting natural enemies and enhancing their predation rate, thus not showing synergetic effects (i.e. greater than the summed effects resulting from each level). Consequently *hypotheses 2, 8 and 10* were not confirmed by our experiments. We observed however interactive effects from the combination of both level of diversification on abundance of arthropods, although the effects were not the same across years. Concerning herbivores, the cultivar mixture hosted higher abundance of aphids than the least susceptible cultivar when grown as a sole crop, but when grown in intercrops, the cultivar mixture hosted lower or higher (depending on the year) abundance of aphids compared to single cultivar. Concerning the natural enemies, predatory ground beetles were found in equal abundance in wheat whatever the level of intraspecific diversity when grown as sole crop, but when intercropped, we observed difference among cultivars.

When taken separately, increasing intraspecific diversity reduced aphids compared to the most susceptible cultivar, but not compared to the least susceptible cultivar, and the same pattern occurred with regard to the attraction of natural enemies. Thus *hypotheses 1 and 7* are not supported for intraspecific diversification. However, increasing interspecific diversity tended to decrease aphids and to attract ground beetles, but no other taxa of natural enemies were clearly attracted. Thus our experiments partially support *hypotheses 1 and 7* for interspecific diversification. Our results further suggest that the effect of interspecific diversification on the community composition of predatory ground beetles is site-dependant. Predation and parasitism rates were not influenced by any diversification practices (*Hypothesis 9* not verified).

Agronomic performances, assessed in terms of cereal grain yield and nitrogen content were not at their highest values in the most diversified treatment, thus not showing synergetic effects from combining diversification practices. Agronomic performances were as good in cultivar mixture as in the monocultivar treatments, with one exception in 2016 where the cultivar mixture yielded 12% less than the best yielding cultivar (Renan). Our experiments partially support *hypothesis 3* for intraspecific diversification. Agronomic performances were steadily negatively impacted by intercropping, with -10% in yield and -7% in grain nitrogen content. We expected such effects concerning the grain nitrogen content, but not for the yield. Thus *hypothesis 3* is also partially supported for interspecific diversification.

Based on our laboratory experiments, we observed that, at individual level, intercropping wheat and clover did reduce aphid host location abilities through delayed in reaching its host plant and reduced residence time on the host plant supporting our *hypothesis 4*. This was however not the case when wheat was intercropped with pea, which confirms the *hypothesis 6*. At the population level, wheat-legume intercrops (i.e. wheat/clover and wheat/pea intercrops) reduced the absolute number of aphids on wheat plants compared to wheat sole crops (*hypothesis 5* verified). If we take into account aphid densities (number of

aphids related to plant biomass), only intercropping wheat with clover significantly reduced aphid densities on wheat (*hypothesis 6* verified). Intercropping wheat with a legume non-host plant was disruptive to the cereal aphid *Sitobion avenae*, but the species used as non-host plants, its biomass and the ratio of host to non-host plants are three important parameters that should be taken into account in studies on intercropping systems.

## **6.2 Ecostacking - increasing the right diversity**

In our study, increasing diversity at the field scale did not result in significantly higher pest regulation. Different reasons can be put forward to explain why increasing diversity *per se* may not result in higher pest control.

### **6.2.1 Increasing diversity *per se* does not necessarily result in higher functionality**

It is recognized that biodiversity positively influence agroecosystem functions (Cardinale et al., 2012), and several studies have demonstrated that increasing diversity of plants and/or natural enemies increase the service of pest control (Letourneau et al., 2009; Ratnadass et al., 2012; Dassou & Tixier, 2016). However, the relationship between diversity and function in general may not be as straightforward as it sounds (Swift et al., 2004). Our study confirms this statement, because we did not observe that increasing plant diversity in crops increased the predation activity of natural enemies within the field, nor reduced significantly the herbivores. Therefore increasing biodiversity *per se* does not necessarily result in higher functionality. Increasing diversity may for example favour negative interactions among plants or natural enemies that may consequently reduce the pest control. Intraguild predation is a good illustration of this phenomenon as it may reduce the impact of natural enemies on herbivore populations, due to the consumption of the predator of the herbivore by another predator (Straub et al., 2008; Letourneau et al., 2009). Intraguild predation between spiders and ground beetles exists in wheat fields (Lang, 2003) and may have occurred in our study.

We observed indeed that only the abundance of ground beetles but not spiders increased significantly in the intercrops, while we may have expected spiders to also increase in the presence of a cover of white clover (Gravesen, 2008). This may be a result of increased predation of ground beetles on spiders, but we can only assume it. Additionally, increased diversity may enhance alternative prey that distract the natural enemies from the targeted pest, instead of sustaining natural enemies within the crop when the level of pests is low (Prasad & Snyder, 2006). In our study, the presence of a cover crop may have favored alternative preys (e.g. springtails) to the detriment of artificial sentinel preys, as for spiders that have been shown to eventually switch from targeted aphid preys to springtails (Gravesen, 2008; Kuusk & Ekbohm, 2010). Competition for resources between plants may also reduce the production function of the agroecosystem. We observed indeed competition between the plants of wheat and white clover, with reduced agronomic performances of the wheat when intercropped compare to wheat monocultures.

Another element advocating for the fact that increasing biodiversity *per se* does not necessarily result in higher functionality, is that the combination of both genetic and species diversity, which was the most diversified treatment in our field experiments, did not result in a higher level of reduction of aphid populations, an enhancement of natural enemy populations or of their predation rate. Our objective of "ecostacking" both levels of diversity relied on the hypothesis that each single level represent an ecosystem service provider and would individually improve the regulation function of pest control. As we found a very limited impact from the genetic level, we can hardly expect additive effects (i.e. equal to the summed effects resulting from each level) to occur when combining both genetic and species levels. We observed however non-additive effects (or interactive effects), that are not predicted by the addition of the responses to genetic and species diversity, but are the result of interactions among the two levels (Johnson et al., 2006). We did however not observe synergetic effects (i.e. greater than the summed effects resulting from each level). As already

mentioned earlier, the studies manipulating both intra- and inter- specific diversity of host plants on herbivores and their natural enemies are very few and cover a restricted range of ecosystems: tree plantations and sand dune systems (Koricheva & Hayes, 2018). This is a brand new area of research for agroecologists, and this work provides the first observations on the potential of combining planned genetic and species biodiversity in annual crops. Among the existing studies reviewed by Koricheva & Hayes (2018), none reported additive effects from combining both level of diversity (Table 6.1), but one observed synergetic effects. This example is interesting because it shows that even if there is no effect from individual diversification practices, the combination of diversification practices may result in the appearance of an effect on arthropod activity (here herbivory).

The network of interaction involved in diversified plant mixtures is enormous. Andow (1991) indicates that "a relatively simple ecosystem of 2 plant species, 6 herbivore species, and 6 natural enemy species has 91 potential two-way and 364 potential three-way ecological interactions and at least an equal number of possible evolutionary responses". It is therefore a hard task to predict how well will do such specific plant mixture compared to another one (Gardarin et al., 2018). In our study, we focused on a specific group of pest (cereal aphids), for strictly applied reasons, because they are the main damaging pest in wheat crops. From a more ecological point of view, the effects of diversification on other pest species such as the wheat midges or cereal leaf beetles could have been different and should be investigated. Instead of investigating the response of taxonomic components to diversification within an agroecological network, it is therefore proposed to provide a more functional description of plant and arthropod communities resulting from diversification (Gardarin et al., 2018).

**Table 6.1: Summary of articles that report the effect of intraspecific diversification practices in cereal crops on herbivores, natural enemies and/or production.**

Reference	Country	System	Plot size	Arthropods response to increase in plant:			Nature of the response
				Genetic diversity	Species diversity	Genetic & Species diversity	
<b>Hahn <i>et al.</i> (2017)</b>	China	Sub-tropical tree plantation	666 m <sup>2</sup>	= herbivory	= herbivory	Genetic ↗ herbivory in Species div.	Synergetic
<b>Moreira <i>et al.</i> (2014)</b>	Mexico	Tropical tree plantation	441 m <sup>2</sup>	= herbivory	= herbivory	= herbivory	NA
<b>Campos-Navarrete <i>et al.</i> (2015)</b>	Mexico	Tropical tree plantation	441 m <sup>2</sup>	= herbivore ↗ predator diversity	↗ herbivore diversity = predator	Genetic or Species ↗ predator div. only at low Species or Genetic div. respectively	Interactive
<b>Crawford &amp; Rudgers (2013)</b>	U.S.A	Fresh water sand dunes	2.25 m <sup>2</sup>	= herbivore = predator	↘ herbivore (only in mixtures of 3 species, not 6) = predator	= herbivore = predator	Interactive

### 6.2.2 Functional traits to regulate pests: a better understanding of multitrophic interactions

More than biodiversity *per se*, it is recently argued that specific traits are determining the functionality of an agroecosystem (Straub et al., 2008; Wood et al., 2015; Perović et al., 2018). Concerning particularly the pest regulation function, species identity of predator had stronger effects than species richness on biological control of aphid pests (Straub & Snyder, 2006; Alhadidi et al., 2018). It means that the composition in the functional traits is more essential to ensure biological control than the number of different predatory species. In our study, we used a mixed approach based on taxonomy and the sole feeding habit according to a priori knowledge, i.e. aphid as pests and generalist natural enemies as control agents, as frequently done in such studies. Concerning natural enemies, several traits have been recognized as important to regulate herbivores such as habitat, diet breadth, hunting strategy and body size (Rusch et al., 2015; Greenop et al., 2018). We observed an overall increase of predatory ground dwelling arthropods, especially ground beetles from interspecific diversification, but it did not translate into higher predation activity. A possible explanation is that other essential traits to regulate herbivores (e.g. hunting strategy, body size) may have not been enhanced by intercropping.

The so-called *functional trait* is "a property, either categorical or continuous, of an individual organism that determines its effect on (effect trait) or response to (response trait) the environment" (Wood et al., 2015). In habitat manipulation, increasing the "right" diversity or the functional diversity may for example target an improvement of the resources needed by natural enemies (e.g. ground cover or floral subsidies) without intensifying the pest damages (Landis et al., 2000; Gardarin et al., 2018). Our study confirms that increased ground cover tended to reduce aphid herbivores and enhanced ground dwelling arthropods, especially ground beetles. Ground cover is therefore a promising functional trait to consider for habitat manipulation. At the genetic level, Barot *et al.* (2017) suggested that heterogeneity in traits describing aerial architecture of wheat cultivar mixtures would increase pest regulation. We



did not observe that heterogeneity in straw length reduced cereal aphids. This trait is therefore unlikely to be crucial for controlling aphid pest in wheat. We observed a difference in susceptibility between the two cultivars, but we did not investigate which other traits may be responsible, and no gene of resistance to cereal aphids was identified in modern hexaploid wheats for European farming systems (Dedryver et al., 2010).

If it is currently difficult to advocate specific management options due to the complexity of interactions network implied in herbivore regulation, studies on diversification practices should tend to better identify which functional traits should be managed (Gardarin et al., 2018; Greenop et al., 2018). It is however important to keep in mind that farmers do not manage traits but species or cultivars, especially concerning the associated biodiversity, as well as abiotic conditions. The challenge lies in proposing strategies of habitat manipulation to farmers that relate appropriate species or cultivars to the targeted function by improving the beneficial interactions among components within a field (Wood et al., 2015).

### 6.2.3 The surrounding landscape - the third level of ecostacking

Herbivore and natural enemy species differ in their dispersal ability. Habitat use and landscape composition surrounding the fields influence species composition and spatial distribution of natural enemies and their role as biological control agent (Bianchi et al., 2006). The landscape surrounding a field is therefore likely to also influence the impact of intrafield diversification practices on arthropods (Hatt et al., 2018). We actually did observe that the composition of the predatory ground beetle community was specific to each site in Chapter 5, and that the composition of the community responded differently to intercropping according to the site. The species assemblage of ground dwelling beetles is a result of many factors such as abiotic soil factors, crop types, but also the presence of non-cropped areas in the landscape (Holland & Luff, 2000). We may therefore consider that the surrounding landscape represents a third level of ecostacking and that it could have a significant influence. Any diversification

practices may interact with the surrounding landscape and the response of arthropods may be different according to the complexity of the landscape.

Indeed, enhancement of local diversity is expected to have lower beneficial impact on biodiversity in complex landscape compared to simple landscapes (Tscharntke et al., 2005). Complex landscapes already benefit from a high biodiversity and a high connectivity that may act as a source of dispersing species towards fields and therefore sustaining populations' persistence after a disturbance (Tscharntke et al., 2005). In our study, we may have benefit from rather rich and complex surrounding landscape. The region and the farms where we worked were characterized by low field size (4.5 ha in average and ranking from 1.8 ha to 15 ha), and a landscape composed at 65% by crop lands. This value may be considered to indicate a rather complex habitat, if we compare to the literature (Thies et al., 2003; Winqvist et al., 2011; Martin et al., 2015). The field size has also a strong influence on biodiversity measures in crop fields, and smaller fields host more diverse and abundant arthropods (Fahrig et al., 2015). The size of the field determines the perimeter-to-area ratio (i.e. the ratio of the perimeter to the area of the cultivated field) and a high perimeter-to-area ratio demonstrates an important connection of the field with its margins (Östman et al., 2001), which represent a source of natural enemies migrating from field margins towards the crops (Denys & Tscharntke, 2002; Tscharntke et al., 2007).

### **6.3 Confounding factors at the field scale influencing diversification practices**

Any ecological and agronomical studies are facing the difficulties to identify and border the influence of elements interacting with the experimental area, which has been artificially delimited by the researcher (Levin, 1992). That is to say, that several elements left aside by our experimental design, or in the studies with which we are comparing our observations, may provide clarifications concerning the variable results on the pest control potential of

diversification practices. The experimental evidences we gathered highlight several points to consider in studies on the potential of diversification practices to promote pest control. We detail below several factors that may confound the interpretation of comparisons among studies on the influence of diversification practices on arthropods and pest control.

### 6.3.1 Organic vs. conventional farming

This parameter is often overlooked in meta-analyses concerning the influence of diversification practices on pest control. Organic and conventional farming should however be considered separately because they do not rely on the same farming practices (e.g. sowing dates, mechanical weeding, fertilization) and have different objectives in term of agronomic performances and biodiversity conservation, including natural enemies (Hole et al., 2005; Mason & Spaner, 2006). Our field experiments were performed under organic farming conditions and aphid pest infestation and their regulation by natural enemies may therefore not be influenced by diversification practices in the same way than under conventional farming conditions.

Very few studies have investigated the difference in pest control between organic and conventional farming systems (Letourneau & Bothwell, 2008). Only recently, a broad meta-analysis reveals that levels of insect pest infestation are similar in organic vs. conventional annual cropping systems, while biological control of animal pest tends to be higher in organic farming (Muneret et al., 2018). This general observation may slightly vary according to specific species. For example, organic wheat crops are characterized by lower inputs and nitrogen deficiency (David et al., 2005; Mason & Spaner, 2006), which is known to negatively impact the development of cereal aphids (Duffield et al., 1997; Aqueel & Leather, 2011). Conventional cereal fields consequently host higher number of aphids compared to organic fields (Reddersen, 1997). This matches with our observations of relatively low level of aphid infestations in our field experiments (Chapter 3). As the effect of intraspecific diversification on pest regulation is known to be influenced by the level of biotic pressure

(Power, 1991), the low level of aphids infestation in our experiments may therefore explain the apparent lack of efficiency of diversification practices in reducing pests.

Additionally, interactive effects may be observed between organic farming and landscape complexity. For example, Schmidt *et al.* (2005) found that the abundance of ground-dwelling spiders was greater in organic winter wheat fields than in conventional, but that landscape complexity increased spider density only in conventional farming. In terms of pest control, results are variable in cereal cropping systems. Winqvist *et al.* (2011) observed that aphid predation rate in conventional farming was high and independent from landscape complexity; while in organic farming it decreased as the landscape became increasingly simplified. On the contrary, Birkhofer *et al.*, (2015) found higher aphid predation in organic farming independently from landscape complexity. This highlights the interest of considering the landscape as a potential third level of ecostacking.

Evenness of natural enemies, rather than species richness, is also promoted by organic farming, and can sustain ecosystem functions such as pest control (Crowder *et al.*, 2010). Similarly to the interaction that exists between landscape complexity, the components and the interactions within organic fields may therefore already be richer and the effect of diversification is therefore less obvious compared to conventional fields. That may be why, we did not observe strong effects from increasing genetic and species plant diversity on pest control. Concerning agronomic performances for example, Kaut *et al.*, (2008) observed that cultivar mixtures of cereals managed under conventional or organic do not perform equally well because they do not face the same constraints, especially in terms of weed pressure (Muneret *et al.*, 2018). Care should therefore be taken before to extrapolate the results to conventional farming systems or when comparing our results with conventional farming systems.

### 6.3.2 Experimental scale

The size of experimental plots is an essential parameter of studies investigating the effect of diversification on pest control. Indeed, it has been shown that the influence of diversification on herbivores and predators differs according to the plot size (Smith & Mcsorley, 2000; Bommarco & Banks, 2003; Letourneau et al., 2011; Dassou & Tixier, 2016). In their meta-analysis, Bommarco & Banks (2003) found that experimental plot size below 256 m<sup>2</sup> had large negative effects on herbivores and, to a lesser extent positive effects on predators, while no effect were observed in experimental plots larger than 256 m<sup>2</sup>. The observations we reported in the prior chapters are therefore consistent with Bommarco & Banks (2003) taking into consideration that we designed experimental plots of 1200 m<sup>2</sup> and such dimensions are closer to reflect real farming conditions than smaller plots. Small plot size may overstate the reduction of herbivores due to diversification. When distance between treatment plots is small, herbivores may aggregate in the plot that is the most concentrated in their host plant, showing a "patch choice" response (Bergelson & Kareiva, 1987). This behaviour skews the interpretation of experiments that compare monoculture vs. polyculture, but most of meta-analyses investigating the impact of diversification on pest control tend to mix-up scales of field experiments (Poveda et al., 2008; Lopes et al., 2016).

### 6.3.3 Design of the diversification practice

The design of the diversification practice it-self, in terms of relative proportion of plants or spatial arrangement, is also a source of variability in the response of arthropods (Ratnadass et al., 2012). In intercropping systems, as highlighted in our laboratory experiment (see Chapter 4), the ratio of host to non-host plants influence the host location behaviour of aphids and may consequently determine their abundance in the fields (Power, 1990). If comparisons of additive (addition of both densities of plants compared to monoculture) vs. substitutive (total density equals the monoculture) designs is sometimes considered in meta-analyses on diversification for pest control (Letourneau et al., 2011; Iverson et al., 2014), to our

knowledge the ratio of host to non-host plants has never been investigated. In their study on pea-wheat intercropping, Ndzana *et al.* (2014) observed that additive row intercrops are significantly more infested by pea aphids than substitutive row intercrops, but the pea (host) to wheat (non-host) ratio is not equivalent with a more important density of non-host plant in substitutive intercrops (3.5 wheat plants for 1 pea plant) compared to additive intercrops (1.9 wheat plants for 1 pea plants). This result confirms what we have found in our study: the ratio of host to non-host plants may be an important parameter to explain the success of interspecific diversification in term of bottom-up control of pest. Consequently, we suggest that diversification practice should be designed with a higher proportion of non-host plant compared to host plant, provided that the production service of the cash crop is not negatively impacted.

Moreover, it is essential to consider that intercrop-monocrop comparisons are biased because plant breeding is almost exclusively oriented towards performance under monoculture conditions (Lithourgidis *et al.*, 2011; Bedoussac *et al.*, 2015; Brooker *et al.*, 2015; Stagnari *et al.*, 2017). In our case for example, we used the cultivar Renan, which is considered as the reference cultivar for French organic wheat farming (Dawson *et al.*, 2013) and another modern cultivar that have been selected to be grown alone. Therefore, the potential of intercropped systems may be underestimated, both in terms of agronomic performances and in the ecosystem services they support. This observation is also true for the design of cultivar mixtures. Specific breeding approaches are needed to select varieties for their abilities to complement each other within a mixture (Barot *et al.*, 2017; Borg *et al.*, 2018). There is also a lack of rules to design cultivar mixtures to provide targeted functions, especially concerning the minimum number of cultivar, the proportion of each cultivar (equal or not) or the traits to select (e.g. ) that may promote pest control (Barot *et al.*, 2017; Borg *et al.*, 2018). In our study, we could only compare two different wheat cultivars due to the design of our experiments. This may not be diverse enough to compose a mixture with a large

heterogeneity of traits. For example, aphid reduction were observed from wheat cultivar mixtures with six different lines but not less (Shoffner & Tooker, 2013). There is therefore a great need to organize the knowledge transfer concerning how efficient is a specific diversification practice in terms of pest control (Gardarin et al., 2018).

## **Chapter 7:**

### Conclusion and perspectives



The biodiversity loss and the homogenization within the agroecosystems are of major concern nowadays. Increasing the diversity of the cultivated biodiversity is proposed as a way to restore the ecological processes that may benefit the functionality of the agroecosystems. By developing the ability of the cropping system to inherently regulate pests, diversification practices pursue the objective to reduce the use of pesticides and their negative impacts. We selected two diversification practices that have good potential to be implemented by farmers because they fulfill some of their needs in term of ecosystem service such as fertilization, disease and weed control. We aimed at investigating their potential for pest control and eventually add this argument to motivate farmers to implement those practices.

However, we have seen that increasing diversity *per se* does not necessarily improve pest control. Ecostacking both genetic and species plant diversity in wheat crops does not promote significantly the reduction of herbivores, nor the increase of natural enemies and their activity of predation within the field it-self. However, we only focused on a small part of the arthropod community. Other pests, other natural enemies, other predator-prey interactions and even other components of biodiversity such as pollinators or granivorous arthropods may also be involved in the system we studied, and may have been impacted in some ways by the increase of crop plant diversity, what we did not monitor. In addition, our studies highlight the importance of multi-year investigation and on large experimental plots. We therefore propose to investigate further the potential of wheat cultivar mixtures and wheat intercropped with clover on a broader range of arthropods for several years and, as done in our study, on large plots, preferably under real farming conditions. Moreover, we worked under organic farming conditions, and extrapolation to conventional farming should be taken with care. We suggest to undertake similar experimental field work under both farming systems to evaluate how different may be the effect of diversification on arthropods and their activity.

It seems that the search for specific functional traits within diversification practices may be more powerful to deliver ecosystem services rather than taxonomic identity. The challenges for agroecologists are therefore to identify the traits present in diverse plant community that are involved in promoting pest control, and to propose combination of plants that may be cultivated to both enhance production and natural pest control.

Additionally, we propose to consider in future studies the landscape as a third level of ecostacking. We are fully aware that combining both level of diversification at the field scale and a significant landscape analysis, including an adequate number of replicates, represent a challenging amount of work. However, research projects regrouping agronomists, entomologists and landscape ecologists could overcome such difficulties. In that respect, the Ecostack project, funded by the European Union, aims at developing "ecologically, economically and socially sustainable crop production strategies via stacking of biodiversity service providers and bio-inspired tools for crop protection, within and around agricultural fields", and could open the door to promising investigation towards an enhancement of the sustainability of agroecosystems across Europe.

This PhD work gives the first insights on which to build experimental field work in order to investigate the potential of ecostacking multiple level of diversity for enhanced pest regulation.

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## Résumé substantiel en français

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### *Introduction*

Depuis le développement de l'agriculture, l'Homme est confronté au problème des ravageurs de culture qui détruisent les récoltes. Si l'utilisation des pesticides a indéniablement permis d'augmenter les rendements à une échelle globale (Tilman et al., 2002), cette amélioration des performances agricoles s'est accompagnée de coûts sanitaires et environnementaux importants (Pimentel, 2005; Mostafalou & Abdollahi, 2013; Annett et al., 2014; Gibbons et al., 2015). Plus particulièrement, l'utilisation des pesticides est responsable du déclin des populations d'arthropodes bénéfiques aux systèmes agricoles, tels que les auxiliaires de culture ou les pollinisateurs (Geiger et al., 2010; Potts et al., 2010; Oliver et al., 2015). Parallèlement, l'intensification de l'agriculture ces dernières décennies est responsable de l'homogénéisation spatiale et temporelle des cultures (Benton et al., 2003) et d'un déclin de la diversité génétique des plantes cultivées (FAO, 1997; Wouw et al., 2009; Tooker & Frank, 2012) et des adventices (Weiner et al., 2001; Fried et al., 2009; Arslan, 2018) pouvant avoir des conséquences sur les populations d'arthropodes (Norris et al., 2000). Ainsi les alternatives aux pesticides sont de plus en plus plébiscitées.

Dans ce contexte, les pratiques agroécologiques s'ancrent dans une approche systémique de la protection des cultures en développant la capacité inhérente de l'agroécosystème à réguler les ravageurs présents et en gardant les méthodes thérapeutiques (ex : biopesticides ou lutte biologique classique) comme solution de dernier recours (Lewis et al., 1997; Nicholls & Altieri, 2004; Birch et al., 2011). Parmi les pratiques agroécologiques, les pratiques de diversification, c'est à dire l'augmentation de la diversité végétale, permet de réguler les populations de ravageurs dans de nombreux agroécosystèmes (Hooks and Johnson 2003; Letourneau et al. 2011; Dassou and Tixier 2016). Cela fait référence à l'hypothèse de « résistance associationnelle » (Tahvanainen & Root, 1972) qui peut être expliqué par deux processus écologiques : la régulation dite « bottom-up » qui concerne la régulation des

ravageurs par les plantes elles-mêmes (soit le niveau trophique inférieur) et la régulation dite « top-down » qui concerne la régulation des ravageurs par leurs ennemis naturels (Gurr et al., 2004). En effet, selon l'hypothèse de la concentration des ressources (Root, 1973) les herbivores spécialistes sont plus enclin de trouver et de se développer sur le leur plante hôte quand celle-ci est en monoculture. Des environnements diversifiés tels que les polycultures sont donc moins favorables aux herbivores, mais plus favorables à leur ennemis naturels grâce à une offre plus abondante de ressources (ex : proies ou hôtes alternatifs, nectar ou pollen) ou d'abris (Gurr et al., 2017).

La diversification de l'agroécosystème peut se réaliser à différentes échelles : du champ aux éléments du paysage (Duru et al., 2015). La manipulation des éléments autour du champ, tels que les bandes fleuries ou les éléments semi-naturels, ont principalement pour objectif de favoriser les ennemis naturels en leur fournissant des conditions favorables à proximité des cultures afin qu'ils viennent réguler les ravageurs des cultures (Gurr et al., 2017). Cependant le recours à ces pratiques par les agriculteurs reste limité car leur efficacité est variable et elles peuvent être considérées comme une perte de surface cultivable (Tscharntke et al., 2016 ; Begg et al., 2017). Au sein du champ, la diversité végétale peut être augmentée à deux niveaux: génétique (diversité intraspécifique) ou spécifique (diversité interspécifique). La première consiste en l'utilisation de mélanges variétaux tandis que la deuxième repose sur les associations de cultures, c'est-à-dire deux cultures de rente ou une culture de rente avec une plante compagne (Andow 1991). Dans ce cas, l'objectif principal est d'optimiser la surface cultivable en associant des variétés ou des espèces complémentaires (Brooker et al., 2015 ; Garibaldi et al., 2017).

### *Problématique*

Dans notre étude nous avons choisi d'étudier l'application de deux pratiques de diversification en cultures céréalières: le mélange de variété de blé (diversification intraspécifique) et l'association du blé avec un couvert de trèfle blanc (diversification interspécifique). Ces

pratiques sont considérées comme des pratiques agroécologiques prometteuses pour les systèmes de culture à bas intrants ou pour l'agriculture biologique car elles favorisent de nombreux services écosystémiques, tels que la régulation des maladies ou des adventices, ainsi que la fertilisation azotée (Finckh et al. 2000; Mundt 2002; Vrignon-Brenas et al. 2018). Or les agriculteurs sont particulièrement susceptibles d'adopter des pratiques délivrant un panel de services écosystémiques (Gurr et al., 2017). Cependant, le potentiel de régulation des ravageurs de culture de ces deux pratiques n'a pas été évalué en conditions réelles de cultures. Plus particulièrement, la combinaison de ces deux niveaux de diversification n'a jamais été étudié en système agricole et très peu en systèmes naturels (Hokkanen & Menzler-Hokkanen, 2018 ; Koricheva & Hayes, 2018). Or le lien entre la biodiversité, définie en tant que nombre d'espèces, de gènes ou de traits fonctionnels, et le fonctionnement des écosystèmes dont les agrosystèmes fait aujourd'hui consensus (Cardinale et al., 2012).

→ Le premier objectif de cette thèse est donc de déterminer l'influence de chaque pratique (intra- et interspécifique) prises séparément et en combinaison sur les pucerons du blé en plein champs.

**Hypothèse 1** : les pucerons seront réduits par chaque pratiques de diversification ;

**Hypothèse 2** : la combinaison des pratiques aura un effet synergique sur la régulation des pucerons ;

**Hypothèse 3** : les performances du blé (rendement et taux d'azote du grain) seront au moins aussi bonnes qu'en monoculture (excepté un léger effet négatif sur l'azote du grain dans le cas de l'association blé-trèfle).

→ Le deuxième objectif de cette thèse est d'évaluer l'impact de deux associations de blé-légumineuses (c'est à dire : blé-trèfle et blé-pois) sur les capacités du puceron à localiser sa



plante hôte dans un contexte diversifié et les conséquences sur le développement des populations de pucerons.

**Hypothèse 4 :** le puceron aura plus de mal à localiser sa plante hôte en association ;

**Hypothèse 5 :** le développement des populations de pucerons seront moindre en association ;

**Hypothèse 6 :** le comportement et le développement des populations sera différents selon l'espèce associée au blé.

→ Le troisième objectif de cette thèse est de déterminer l'influence de chaque pratique (intra- et interspécifique) prises séparément et en combinaison sur les ennemis naturels des pucerons et leur activité de prédation.

**Hypothèse 7:** l'abondance des auxiliaires sera augmentée par chaque pratiques de diversification ;

**Hypothèse 8:** la combinaison des pratiques aura un effet synergique sur l'abondance des auxiliaires ;

**Hypothèse 9:** la prédation de proies sentinelles et le parasitisme des pucerons seront augmentés par chaque pratiques de diversification ;

**Hypothèse 10:** la combinaison des pratiques aura un effet synergique sur a prédation de proies sentinelles et le parasitisme des pucerons.

*Mélanges de variété de blé et association avec une plante de couvert pour contrôler les pucerons du blé.*

L'objectif de ce chapitre est d'estimer le potentiel de chaque pratique individuellement pour réguler les ravageurs, mais également lorsqu'elles sont combinées ensemble. Nous avons mené des expérimentations en conditions réelles de cultures et sur deux saisons 2015-16 et 2016-17, afin d'examiner les impacts de ces pratiques de diversification sur les populations de pucerons. Nous avons réalisé un suivi des populations de pucerons présents sur le blé. Nous

avons également caractérisé le couvert végétal (couverture du sol et hauteurs de blé) car une plus grande hétérogénéité peut influencer les pucerons (Bottenberg and Irwin 1992; Barot et al. 2017). Enfin, nous avons mesuré les performances du blé en terme de rendement et de qualité du grain, qui représentent des paramètres déterminants pour l'adoption de ces pratiques par les agriculteurs.

En ce qui concerne la régulation des pucerons, nous n'observons pas d'effets synergiques résultant de la combinaison des pratiques de diversification génétique (mélange de variété de blés) et spécifique (association blé et couvert de trèfle). C'est-à-dire que l'abondance des pucerons n'est pas inférieure lorsque les pratiques sont combinées par rapport aux pratiques prises séparément. Les populations de pucerons tendent à être moins importantes dans l'association blé-trèfle comparativement à la monoculture de blé, tandis que pour le mélange de variété, les résultats sont intermédiaires. Nous observons une variation inter-annuelle de l'apparence des pics de pucerons et des effets des pratiques de diversification ainsi que des variations climatiques qui ont impacté le développement du blé et du trèfle. Les performances agronomiques (rendement et taux d'azote dans les grains) sont réduites dans le cas de l'association blé-trèfle, tandis qu'elles ne sont pas impactées par le mélange de variété de blé. Nos résultats suggèrent qu'augmenter la diversité cultivée au sein du champ peut aider à réguler les pucerons dans une certaine mesure, mais la combinaison des pratiques de diversification génétique et spécifique ne résultent pas en un trade-off entre la régulation des ravageurs et les performances agronomiques particulièrement attractifs pour les agriculteurs.

*L'association de culture modifie les capacités du puceron du blé (Hemiptera : Aphididae) à localiser sa plante hôte ainsi que le développement des populations.*

Selon plusieurs hypothèses, la présence d'une plante compagne pourrait diminuer la capacité du puceron à localiser et à coloniser sa plante hôte (Vandermeer, 1989; Finch & Collier, 2000; Poveda et al., 2008). Dans ce chapitre, nous comparons la capacité du puceron du blé *Sitobion avenae* (F.) à localiser sa plante hôte dans une monoculture de blé (*Triticum*

*aestivum* L.) et dans deux associations de culture différentes: le blé associé au trèfle blanc (*Trifolium repens* L.) – notre modèle d'étude, et le blé associé au pois (*Pisum sativum* L.). Ces deux associations sont des pratiques utilisées par les agriculteurs (Lopes et al., 2016; Vrignon-Brenas et al., 2018) et les associations de céréales et de légumineuses ont montré leur capacité à réduire les pucerons en plein champ (Ndzana et al., 2014; Lopes et al., 2015; Hatt et al., 2018). Ces deux plantes compagnes sont aussi structurellement différentes. Le pois est caractérisé par la production de vrilles ainsi qu'une hauteur supérieure à 60cm (Cousin, 1997; Bedoussac & Justes, 2010b), tandis que le trèfle blanc se développe d'avantage horizontalement et n'atteint pas plus de 20-30cm de hauteur (Frame & Newbould, 1986; Frame, 2005). En plein champ, ces deux plantes sont semées à différentes densités lorsqu'elles sont associées au blé.

Dans ce chapitre nous avons réalisé des expériences comportementales pour vérifier si la capacité du puceron à localiser sa plante hôte est réduite en présence d'une plante compagne, et si cela diffère selon l'espèce de la plante compagne. Nous avons également observé la croissance des populations de pucerons selon la présence ou non d'une plante compagne et selon l'espèce. Nos résultats indiquent que les pucerons ont mis plus de temps à localiser leur plante hôte et ont passé moins de temps dessus lorsque le blé était associé au trèfle comparativement au blé en monoculture ou associé au pois. Il semblerait que cet effet soit principalement dû à la différence de densité entre les deux types d'association, le trèfle étant plus densément peuplé que le pois. De plus, associer du blé à une légumineuse a réduit le nombre absolu de pucerons sur les plantes de blé comparativement au blé cultivé en monoculture. Cependant, nous avons observé que si nous prenions en compte la biomasse des plantes, la densité de pucerons (nombre d'individus par gramme de plante hôte) était significativement inférieure seulement dans le cas de l'association blé-trèfle.

Ainsi nos observations suggèrent que le ratio entre les plantes hôtes et les plantes compagnes est un paramètre explicatif du succès d'une association de culture pour réguler les ravageurs.

Augmenter le temps que le puceron met à localiser une plante hôte, peut entraîner des coûts énergétiques diminuant les capacités reproductrices des individus (Stearns, 1992), et avoir des conséquences sur le développement des populations de ravageurs (Hooks & Fereres, 2006). Nous suggérons donc que les pratiques de diversifications interspécifiques doivent comporter une proportion plus grande de plantes compagnes que de plantes hôtes, à condition que cela n'impacte pas le service de production de la culture de rente. En effet, une compétition entre les deux plantes associées peut réduire la biomasse de la plante hôte, comme nous l'avons observé dans le cas de l'association blé-pois. Alors, la réduction du nombre absolu de pucerons peut être d'avantage lié à une réduction de la matière végétale dont il se nourrit plutôt que d'un effet comportemental (Bukovinszky et al., 2004). Il est donc important de considérer la densité de pucerons en rapport avec la biomasse de sa plante hôte pour déterminer l'efficacité d'une association de culture à réguler les ravageurs.

*Combiner diversification intra- et interspécifique pour améliorer la lutte biologique par conservation des champs de blé*

L'augmentation de la diversité au sein du champ peut permettre de réguler les ravageurs de culture. Dans le chapitre précédent nous nous sommes intéressés particulièrement au mécanisme de régulation des ravageurs par les plantes (bottom-up control). Un autre mécanisme peut également intervenir dans la régulation des ravageurs quand ceux-ci sont contrôlés par leurs prédateurs: le "top-down control". En effet, des environnements diversifiés sont supposés être plus attractifs et bénéfiques aux auxiliaires de culture (hypothèse des ennemis naturels; Root, 1973), grâce à une plus grande abondance d'abris, de nectar, de pollen et de proies alternatives (Gurr et al., 2017). Dans ce chapitre nous nous intéressons à nouveau à l'application de deux pratiques de diversification en cultures céréalières: le mélange de variété de blé (diversification intraspécifique) et l'association du blé avec un couvert de trèfle blanc (diversification interspécifique). L'influence de la diversification intraspécifique sur les ennemis naturels a été relativement peu étudiée, mais quelques études tendent à montrer des

effets positifs en culture céréalière (Ninkovic et al., 2011; Chateil et al., 2013; Grettenberger & Tooker, 2017). Les associations de cultures incluant une plante de couvert, tel que le trèfle dans notre modèle, peuvent fournir des abris et des microclimats attractifs aux ennemis naturels au sein du champ (Booij et al., 1997; Carmona & Landis, 1999; Wilkinson, T. K., & Landis, 2005; Schmidt et al., 2007; Lundgren & Fergen, 2010). Cependant, la seule présence d'ennemis naturels ne garantit pas la régulation des ravageurs (Furlong & Zalucki, 2010). Il est donc nécessaire d'inclure des mesures de l'activité de prédation des ennemis naturels présents dans notre dispositif expérimental. Pour cela nous avons recours à des proies sentinelles réelles (carte de prédation) au niveau du feuillage du blé et artificielles (chenilles en pâte à modeler) au sol, qui permettent d'évaluer les différences de pression de prédation dans nos différentes modalités expérimentales (Lövei & Ferrante, 2017).

Nous avons observés que les ennemis naturels épigés, particulièrement les carabes prédateurs, étaient relativement plus abondants en présence d'un couvert de trèfle. Le mélange de variété de blé ne semble pas avoir d'effets particuliers sur les ennemis naturels. Les observations varient selon les arthropodes et selon leur position au sein du couvert végétal (sol ou feuillage). Nous avons également observés que la composition spécifique des communautés de carabes prédateurs semblent fortement influencée par le couvert de trèfle et le site d'échantillonnage, indiquant même une interaction entre les deux facteurs. Cependant, le taux de prédation des proies sentinelles et le taux de parasitisme n'est pas impactés par les pratiques de diversification.

Nos observations confirment l'intérêt de l'association avec des plantes de couvert telles que le trèfle pour favoriser les auxiliaires actifs au niveau du sol, mais ne semblent que peu attractifs pour les auxiliaires des strates supérieures. L'absence de suppléments floraux peut avoir limité l'attractivité du couvert pour certains auxiliaires tels que les syrphes ou les guêpes parasitoïdes. Bien que l'effet des mélanges de variétés de blé sur les auxiliaires soit assez peu étudié, certaines études ont pu montré un effet positif sur certaines araignées (Linyphiidae) (Chateil et al., 2013 ; Dubs et al., 2018). Nos observations confirment la nécessité d'avoir

recours à des méthodes d'évaluation de la pression de prédation exercée par les ennemis naturels en champs. Il est possible que l'augmentation de la diversité cultivée ait permis l'apparition de nouvelles interactions trophiques limitant l'efficacité des pratiques à favoriser la régulation des ravageurs par les ennemis naturels, telles que la prédation intragilde qui peut se mettre en place entre les carabes et les araignées, mais aussi la distraction par des proies alternatives (Lang, 2003 ; Prasad et al., 2006 ; Gravesen, 2008).

### *Discussion*

Si l'on reprend les principaux résultats observés dans nos expérimentations en champ, la combinaison de la diversité intra- et interspécifique n'offre pas de meilleurs résultats en ce qui concerne la régulation des pucerons ou l'attractivité des auxiliaires de cultures et leur activité de prédation. Le mélange de variété de blé n'était pas moins infesté que la variété la plus résistante. On observe plutôt un effet de dilution des résistances au puceron. En laboratoire, seuls les mélanges de variété de blé contenant six variétés différentes ont permis de réguler les populations de pucerons comparativement à des mélanges moins diversifiés (Shoffner & Tooker, 2013). Concernant la diversité interspécifique, nos résultats indiquent globalement des abondances moins importantes de pucerons sur le blé associé au trèfle comparativement à du blé en monoculture, mais les performances du blé sont également réduites. Cela signifie que la baisse du puceron peut d'avantage être liée à une baisse de qualité de sa plante hôte plutôt qu'à un autre mécanisme de régulation bottom-up. Les associations de blé avec une plante compagne ont montré une grande variabilité concernant leur efficacité à réguler les ravageurs avec seulement 50 % de succès (Lopes et al., 2016).

Ainsi, augmenter la diversité *en soi* ne se traduit pas forcément par une augmentation de la fonctionnalité (Swift et al., 2004). L'augmentation de la biodiversité peut favoriser les interactions négatives (ex : prédation intragilde, compétition, ...) ou des interactions redondantes (ex : partage de niche, distraction par des proies alternatives, ...) (Straub et al., 2008). Il est extrêmement difficile d'évaluer l'ensemble des interactions qui sont en jeu dans

les systèmes de cultures diversifiés (Andow, 1991). Plutôt que la biodiversité *en soi*, la diversité des traits fonctionnels est de plus en plus invoquée pour expliquer le succès des pratiques de diversifications à promouvoir certaines fonctions de l'agroécosystème dont particulièrement la régulation des ravageurs (Gardarin et al., 2018 ; Greenop et al., 2018 ; Perovic et al., 2018). En ce qui concerne les ennemis naturels par exemple, certains traits tels que l'habitat, la stratégie de chasse ou la taille, sont des traits déterminant pour réguler les herbivores (Rusch et al., 2015 ; Greenop et al., 2018). Dans notre étude, la couverture du sol semble être un trait déterminant pour réduire les pucerons et augmenter les effectifs d'ennemis naturels épigés, particulièrement les carabes prédateurs. Il est aujourd'hui essentiel d'arriver à déterminer et à augmenter les traits des mixtures végétales mais aussi des ennemis naturels en interaction avec ces mixtures qui sont responsables de la régulation des ravageurs (Gardarin et al., 2018 ; Greenop et al., 2018). Malgré tout, les agriculteurs ne manipulent pas les traits mais bien des espèces et des variétés et il est donc essentiel d'identifier les traits responsables de la régulation des ravageurs et de les rattacher aux espèces ou aux variétés manipulables par les agriculteurs (Wood et al. 2015). Enfin, le paysage pourrait être considéré comme un troisième niveau de diversité car il influence la composition et la distribution des ravageurs et des auxiliaires (Bianchi et al., 2006). Il est donc probable qu'il puisse influencer l'impact des pratiques de diversification à l'échelle du champ sur les arthropodes (Hatt et al., 2018). Nous avons en effet observé que la composition des communautés de carabes prédateurs était spécifique à chaque site d'échantillonnage dans le Chapitre 5, et que la composition des communautés répondait différemment à l'association blé-trèfle selon le site d'échantillonnage. En effet, la composition spécifique des carabes est le résultat de plusieurs facteurs tels que les facteurs abiotiques du sol, les types de cultures, mais aussi la présence d'éléments semi-naturels dans le paysages (Holland & Luff, 2000). Ainsi, augmenter la diversité locale aurait moins d'impacts bénéfiques sur la biodiversité au sein d'un paysage déjà riche comparé à des paysages pauvres en biodiversité (Tschardt et al., 2005). En effet des paysages riches bénéficie d'un haut niveau de biodiversité et de connectivité qui peut

favoriser les mouvements des espèces vers les champs et maintenir les populations au sein du champ après une perturbation (Tscharntke et al., 2005). Dans notre étude, nous avons ainsi bénéficié d'un paysage relativement riche et complexe avec des champs de petites tailles et des paysages composés à 65 % en moyenne de cultures (Thies et al., 2003 ; Winqvist et al., 2011 ; Martin et al., 2015). La taille du champ a une forte influence sur les mesures de biodiversité en plein champ et des champs de petites surface présentent des communautés d'arthropodes plus diverse et plus abondantes (Fahrig et al., 2015).

Un certain nombre de facteurs limitant pourraient influencer les résultats observés sur les pratiques de diversifications dans cette étude. Premièrement, le contexte de l'agriculture biologique est un facteur très souvent absents des revues de littérature faisant l'état des connaissances sur les pratiques de diversifications et leur influence sur les arthropodes. Hors l'agriculture biologique repose sur des pratiques spécifiques en terme de fertilisation, contrôle mécanique des adventices, dates de semis, ... et présente des objectifs différents en terme de performances agronomiques et de conservation de la biodiversité (Hole et al., 2005 ; Mason & Spaner, 2006). Par exemple, l'agriculture biologique favorise une diversité et une biomasse d'adventices généralement plus importantes qu'en agriculture conventionnelle et cela peut influencer la composition et la distribution des ravageurs et des auxiliaires (Muneret et al., 2018). Plus particulièrement, la culture du blé en agriculture biologique est souvent caractérisé par des déficiences en azote (David et al., 2005), qui peuvent impacter le développement des pucerons des céréales (Duffield et al., 1997 ; Aqueel & Leather, 2011). Cela est cohérent avec les faibles niveaux d'infestation observés dans notre étude (Chapitre 3). De plus, il existe des effets d'interactions entre l'agriculture biologique et la richesse du paysage en ce qui concerne les ennemis naturels et la prédation des pucerons (Schmidt et al., 2005 ; Winqvist et al., 2011). Deuxièmement, l'échelle expérimentale est un facteur important des études sur l'effet des la diversification sur la régulation des ravageurs. Par exemple, Bommarco & Banks (2003) observent que des dispositifs expérimentaux < 256 m<sup>2</sup> exacerbe les effets des pratiques de diversification sur les herbivores et les ennemis naturels. Dans



notre étude, nous observons des effets peu marqués des pratiques de diversifications et cela est cohérent avec Bommarco & Banks (2003) car notre dispositif de 1200 m<sup>2</sup> tend à se rapprocher le plus possible des conditions réelles de culture. Hors la plupart des revues de littérature à ce sujet ont tendance à mélanger les échelles d'expérimentations et cela pourrait expliquer l'importante variabilité qui est rapportée. Enfin, l'arrangement spatial et les proportions relatives des plantes associées sont une autre source importante de variabilité concernant l'impact des pratiques de diversification sur la régulation de ravageurs (Ratnadass et al., 2012). Comme souligné dans notre étude en laboratoire, le ratio plante hôte – plante compagne impacte le comportement des pucerons et peut déterminer leur abondance en champ (Power, 1990). C'est un paramètre peu étudié par rapport à l'arrangement spatial (Letourneau et al., 2011 ; Iverson et al., 2014).

Augmenter la diversité *en soi* n'améliore pas forcément la régulation des ravageurs. Combiner la diversité intra- et interspécifique dans les champs de blé ne réduit pas significativement les herbivores et n'augmente pas significativement l'ensemble des ennemis naturels et leur activité de prédation au sein du champ. Cependant, cette étude se concentre seulement sur certaines espèces de ravageurs et d'auxiliaires et parmi la grande complexité des interactions en jeu dans les systèmes diversifiés, d'autres proies, ennemis naturels ou interactions prédateurs-proies pourraient avoir été impacté. Notre étude souligne l'importance d'étudier plusieurs cycles de culture et dans des conditions réelles de culture à grande échelle. Il est proposé d'approfondir le potentiel des mélanges de variété de blé et de l'association avec le trèfle sur d'autres arthropodes et sur plusieurs années, mais aussi de comparer ce type dispositif en agriculture conventionnelle et biologique. Plus que l'identité taxonomique, la détermination des traits fonctionnels responsables de la régulation des ravageurs semble essentielle pour les futures recherches sur les pratiques de diversifications ayant pour objectif la régulation des ravageurs. Enfin, l'intégration de la richesse du paysage comme troisième niveau de diversification semble également une perspective essentielle à ce genre d'étude, bien que cela représente un important travail de terrain.

**Title :** Intra- and intercrop diversification in cereal cropping and effect on pest control

**Keywords :** Agroecology; Pest control; Conservation Biological Control ; Variety mixtures; Cover crop

**Abstract :**

Increasing intrafield plant diversity has been shown to regulate pest populations in various agroecosystems. Polyvarietal mixtures of a crop species (intraspecific diversity) or associations of a crop and a companion plant (interspecific diversity) are both considered as promising agroecological practices for low-input or organic agriculture systems by providing several ecosystem services such as pest, disease and weed control, and nitrogen fertilization. However, combining both diversification practices has not been studied yet in perspective of winter wheat pest control.

In organic field experiments over two growing seasons, we combined both practices and examined the direct impact on aphid and natural enemy populations and on wheat production. We also investigated the potential pest regulation service through the assessment of the rate of predation by using sentinel preys.

Results show that combining intra- and interspecific diversity did not outperform each practice individually in reducing aphid populations, thus not clearly showing synergetic effects. Taken separately, intercropping tended to have lower aphid infestation, while the cultivar mixtures was more infested by aphids than the least susceptible cultivar. Yearly variation in climatic conditions strongly impacted wheat and clover development, as well as the appearance of aphid peaks. Wheat yields and grain nitrogen content were reduced in intercropping by 7 to 10%, but not in cultivar mixtures. Functional biodiversity, especially natural enemies such as ground beetles, tended to be positively correlated to the presence of a clover cover in the wheat fields (interspecific diversification), but did not respond to the wheat cultivar mixture (intraspecific diversification). Results varied according to the family of arthropods concerned and their position within the vegetation layer (ground dwelling or foliage dwelling arthropods). The cover of white clover and the field context influenced the community composition of predatory ground dwelling beetles. Rates of predation on sentinel preys were not influenced by any of the diversification practices.

Under laboratory conditions, we evaluated how combining wheat and legumes (clover or pea) modifies the behaviour of the cereal aphid *Sitobion avenae* in terms of host-plant location, and population growth. We observed that aphids' residence time on wheat was decreased when this host-plant was intercropped with clover. At the population level, wheat-legume intercrops reduced the number of aphids on wheat plants compared to wheat sole crops but if we take into account plant biomass, only intercropping clover with wheat significantly reduced aphid densities on wheat. The species used as non-host plants and their density are important parameters that should be taken into account in studies on intercropping systems and that may explain the large variability in the results observed in the literature.

Our findings suggest that intrafield diversification may regulate wheat aphids to some extent, but combining the two diversification practices did not result in an interesting trade-off between pest regulation and wheat production in real farming conditions.

**Titre :** Diversité intra- et interspécifique dans les systèmes céréaliers et ses effets sur la régulation des ravageurs.

**Mots-clés :** Agroécologie; Régulation des ravageurs; Lutte biologique par conservation ; Mélanges variétaux; Couverts végétaux

### **Résumé :**

Augmenter la diversité végétale au sein même du champ permet de réguler les populations de ravageurs dans de nombreux agroécosystèmes. Les mélanges variétaux (diversité intraspécifique) ou les associations de cultures avec une plante compagne (diversité interspécifique) sont considérées comme des pratiques agroécologiques prometteuses pour les systèmes de culture à bas intrants ou l'agriculture biologique. En effet, ces pratiques favorisent de nombreux services écosystémiques tels que la régulation des ravageurs, des maladies ou des adventices, ainsi que la fertilisation azotée. Cependant, le potentiel de régulation des ravageurs du blé par la combinaison de ces deux pratiques de diversification n'a pas encore été étudié.

Nous avons combiné ces deux pratiques dans le cadre d'expérimentations menées en plein champ et sur deux saisons de culture, afin d'examiner leurs impacts sur les populations de pucerons et d'ennemis naturels. Nous avons également évalué le potentiel de régulation des ravageurs en mesurant les taux de prédation de proies sentinelles.

La combinaison des diversités intra- et interspécifique n'est pas plus performante pour réduire les populations de pucerons que les pratiques prises séparément. L'association de culture blé-trèfle tend à être moins infestée par les pucerons, tandis que le mélange variétal est plus infesté que la variété la moins sensible. Les variations annuelles des conditions climatiques impactent fortement le développement du blé et du trèfle, ainsi que la date d'apparition du pic de puceron. Le rendement du blé, ainsi que le taux d'azote du grain sont réduits par l'association de culture par 7 à 10%, mais pas par le mélange variétal. La présence d'un couvert de trèfle dans les champs de blé, semble avoir favorisé la biodiversité fonctionnelle, particulièrement les ennemis naturels tels que les carabes, mais pas le mélange variétal. Les résultats sont variables selon la famille d'arthropodes concernée et leur position au sein du couvert végétal (au sol ou dans le feuillage). Le couvert de trèfle et le champ ont influencé la composition de la communauté de carabes prédateurs. Les taux de prédation des proies sentinelles n'ont pas été impactés par les pratiques de diversifications.

En laboratoire, nous avons évalué comment l'association du blé avec des légumineuses (trèfle ou pois) pouvait modifier le comportement du puceron du blé *Sitobion avenae* en terme de location de sa plante hôte et du développement de la population. Les pucerons ont résidé moins de temps sur le blé quand il était associé à du trèfle. Les populations de pucerons se sont moins développées dans les associations du blé avec une légumineuse par rapport à du blé seul, mais si l'on prend en compte la biomasse du blé, seulement l'association blé-trèfle a considérablement réduit les densités de pucerons sur le blé. Ainsi l'espèce associée et sa densité sont des paramètres importants qui devraient être pris en compte dans les études sur la diversité interspécifique, car ils pourraient expliquer la grande variation dans les résultats rapportés par les analyses bibliographiques.

Nos résultats suggèrent qu'augmenter la diversité cultivée au sein du champ peut aider à réguler les pucerons dans une certaine mesure, mais la combinaison des deux pratiques de diversification ne résultent pas en un trade-off entre la régulation des ravageurs et les performances agronomiques particulièrement attractifs pour les agriculteurs.