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# Can intercrop control aphid populations and their ability to spread non-persistently transmitted virus?

Sidonie Loïez

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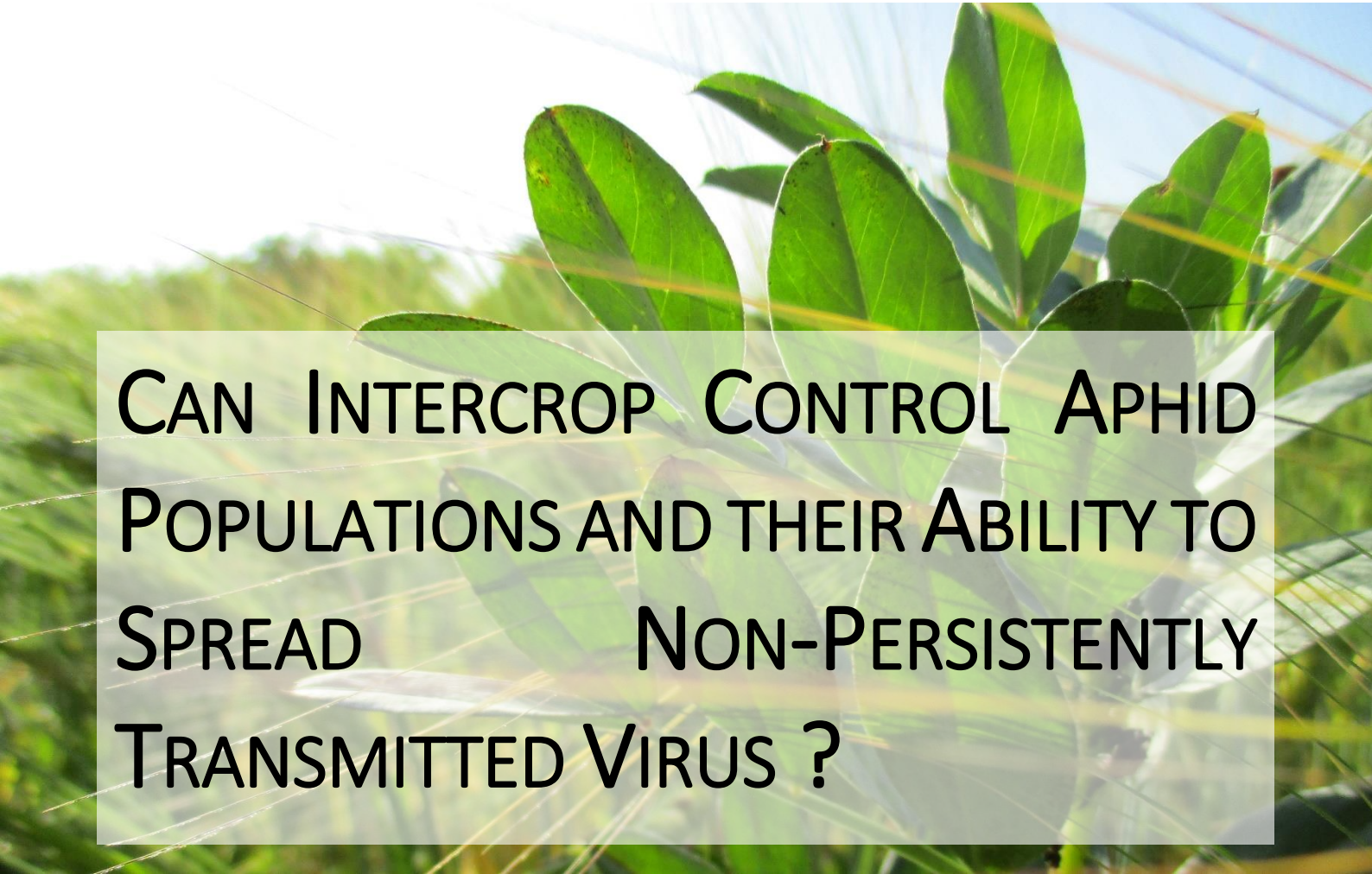
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# CAN INTERCROP CONTROL APHID POPULATIONS AND THEIR ABILITY TO SPREAD NON-PERSISTENTLY TRANSMITTED VIRUS ?

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Insects, in particular aphids, can cause important damage to crops. Until now, chemicals are the most efficient, and widely used method to reduced infestation. Yet, new regulations require good environmental practices, to protect soil and human health. To comply with these, the introduction of higher crop diversity within one field might be a solution. Intercrops (i.e. association in time and space of different crops in a field) have mostly been studied for its agronomic advantages (increase in yield, enhancement of soil properties, economic benefits). However, only a few studies have focussed on the impact of intercrops towards pests. In the present study, implemented in the SRUC-Aberdeen campus, field and greenhouses experiment were carried out on the ability of intercrop to either reduce aphid infestation or non-persistent virus transmission. Barley-legume intercrops were compared to a barley sole crop. The system tested both plant species (bean or pea) and arrangement (row or mix) toward *Sitobion avenae*. All intercrops showed a 30% reduction in aphid numbers during the whole experiment. Intercrops with pea were more efficient to reduce *S. avenae* numbers at the development of the colonies. However, no distinction between the intercrops was observed at the peak of the colonies. Although a higher number of beneficial was found in the barley sole crop, intercrops seem to be efficient to control aphid development in the field. PVY transmission from *Myzus persicae* in a potato-barley intercrop showed no differences compare to sole potatoes. In this system intercrop was not efficient to reduce virus spreading.

Key-words: Intercrops, cereal-legume, *S. avenae*, virus transmission, PVY, *M. persicae*

Les insectes, en particulier les pucerons, sont responsables d'importants dégâts dans les cultures. Jusqu'à maintenant, les insecticides sont le moyen de lutte le plus efficace et le plus utilisé. Cependant, des réglementations récentes imposent de bonnes pratiques pour préserver la santé humaine et celle des sols. Pour y répondre, l'introduction d'une plus grande diversité culturale au champ pourrait être une solution. L'association culturale (AC ; culture simultanée de plusieurs plantes dans un même champ) a majoritairement été étudiée pour ses bénéfices agronomiques (augmentation des rendements, amélioration des propriétés des sols, avantages économiques). Mais très peu ont étudié leurs impacts sur les ravageurs. Dans cette étude menée au SRUC-Aberdeen campus, des expérimentations en champ et en serre pour déterminer les capacités des AC à réduire l'infestation par les pucerons et/ou la transmission de virus non-persistant. Des AC orge-légumineuses étaient comparées à une culture d'orge. Le système testait l'effet de l'espèce associée (féverole ou pois) et de l'arrangement (en ligne ou aléatoire) sur *S. avenae*. Toutes AC montraient une réduction du nombre de pucerons de 30%, sur la période d'étude. Lors du développement des colonies, les AC comprenant du pois étaient plus efficaces à réduire le nombre de *S. avenae*. Au pic des colonies, aucune distinction entre les AC n'a été observée. Malgré un plus grand nombre d'auxiliaires dans les cultures d'orge, les AC semblent efficaces pour contrôler le développement des pucerons en champs. La transmission du PVY dans une association pomme de terre-orge, par *M. persicae*, n'a pas montré de distinction par rapport à un système unique de pommes de terre. Dans ces systèmes, l'AC ne permettait pas de réduire efficacement la diffusion du virus.

Mots clés : Association culturale, céréales-légumineuses, *S. avenae*, transmission de virus, PVY, *M. persicae*

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# Résumé du projet

Les insectes sont une cause majeure de la perte de rendement au champ. Peu de moyens de lutte existent, et l'utilisation de produits chimiques est à ce jour le plus efficace. Toutefois, la réglementation actuelle agricole recommande l'utilisation de pratiques respectueuses de l'environnement.

Des études sur le rôle de la biodiversité en agriculture ont montré qu'il est possible d'améliorer les services écosystémiques, notamment par l'introduction de plus de diversité végétale. C'est pourquoi l'effort de recherche s'intensifie au sujet des associations culturales. Les bénéfices agronomiques de la culture simultanée dans le temps et dans l'espace, de deux – ou plus – espèces végétales sont maintenant connus.

Les associations culturales améliorent par exemple la production générale de la culture et, si ce n'est pas la quantité, c'est la qualité qui est améliorée. De plus, les associations culturales assurent le maintien de bonnes propriétés des sols avec une meilleure rétention de l'eau, une plus grande concentration en matière organique, mais également le transfert d'azote dans le cas des associations céréales-légumineuses. De manière générale, les associations culturales sont plus efficaces que les cultures pures.

En revanche, peu d'étude de ces associations s'intéressent à leurs effets sur les infestations par les pucerons. Pourtant le comportement de ces ravageurs laisse à penser que les associations culturales pourraient être un moyen de lutte contre le puceron.

En effet, les pucerons sont de petits insectes herbivores. Ils se nourrissent de sève, qu'ils aspirent par leur stylet inséré jusqu'aux cellules du phloème. Lorsqu'ils se nourrissent les pucerons affaiblissent leur plante hôte. Ainsi, lorsqu'ils sont présents en grand nombre dans un champ cultivé, ils mettent en péril la production.

Toutefois, les connaissances sur la biologie du puceron ont permis de mieux comprendre leurs interactions avec leur plante hôte. Lorsqu'ils migrent les pucerons sont capables de localiser leur hôte, via l'analyse de stimuli sensoriels principalement. La couleur d'une culture est importante, tout autant que le contraste entre la plante et le sol. Ainsi, il est plus difficile pour les pucerons de localiser une plante hôte dans un couvert dense. Ensuite, les composés volatiles émis par les plantes sont reconnus par les pucerons. Ils sont attirés par leur plante hôte, et généralement repoussés par des non-hôtes. Cependant, des mélanges de ces composés volatiles sont plus difficilement analysés.

Dans leur environnement les pucerons interagissent avec leurs plantes hôtes et leurs ennemis naturels. Ces ennemis peuvent être prédateurs, parasitoïdes ou parasites, certains parmi eux étant également insectes. Parmi les insectes prédateurs, les larves de coccinelle (Coléoptère) et



celles de syrphes (Diptère) sont efficaces. Dans la famille des parasitoïdes se sont principalement des guêpes (Hyménoptère) qui parasitent les pucerons. Elles pondent dans le corps du puceron et leur larve s'y développe en se nourrissant du corps de leur hôte.

Plusieurs hypothèses sur l'efficacité des associations culturales à contrôler le développement de colonies de pucerons émergent. La première est l'hypothèse de « la concentration en ressources ». Les cultures pures seraient plus attractives pour les pucerons que les associations culturales, elles présentent une surface homogène de leur plante hôte. Il est facile pour un puceron de localiser cette plante hôte, de la sélectionner et de s'y développer. Le cas contraire s'observerait en association culturale.

L'hypothèse des « ennemis naturels » prévoit une plus grande fréquentation des associations culturales par les ennemis naturels par rapport aux cultures pures. Ce qui pourrait s'expliquer par des propriétés favorables au développement d'insectes (nombreux habitats, ressources en pollen et nectar) dans les associations végétales. . Et le bénéfice de leur action devrait s'observer par une infestation réduite en comparaison avec une culture pure, moins propice au développement de ces insectes.

De plus, les pucerons sont vecteurs de virus, ce qui les rend d'autant plus néfastes aux regards des agriculteurs. Lors de la recherche de leur hôte, ils réalisent de nombreuses ponctions d'essais. Elles sont brèves mais suffisantes pour transmettre un virus à la plante. Certains de ces virus sont dits non-persistants. Cela fait référence à leur mode de transmission. Une fois acquis par leur vecteur, ce dernier ne peut transmettre le virus que pour une courte période.

Ainsi, l'hypothèse de « perte de transmissibilité d'un virus » s'appliquerait pour les virus non-persistants dans les associations culturales. Les pucerons infectés dans un mélange mettraient plus de temps à trouver leur hôte et donc perdraient leur capacité à transmettre le virus. Ou pendant leur recherche d'un hôte, les pucerons réaliseraient des ponctions d'essai sur la culture non hôte et donc perdraient le virus.

Dans ce contexte, l'étude de l'efficacité des associations culturales à réduire les infestations de pucerons ou la transmission de virus non-persistant a été réalisée. Deux types d'expérimentations ont été mises en place au SRUC-Aberdeen campus. Dans un premier temps des expérimentations en champ ont permis d'étudier les populations de pucerons dans des couverts d'associations culturales. Ensuite des expérimentations en serres, ont permis d'étudier la transmission d'un virus non-persistant.

Au champ, le développement du puceron des céréales *Sitobion avenae*, a été observé dans différentes associations culturales.

- Deux associations en ligne entre orge et féverole ou orge et pois.
- Deux associations en mélange entre orge et féverole ou orge et pois.

Ces observations étaient comparées à une culture pure d'orge.

Cinq répétitions de ces systèmes ont été établies en champs. 30 *S. avenae* ont été introduits au centre de chacun des plots, pour le début de l'expérience. Puis les pucerons étaient comptés deux fois par semaine pendant deux mois.

Les résultats ont montré que le nombre de pucerons dans tous les systèmes d'association culturale était réduit par rapport à celui déterminé en culture pure. En début de saison l'association orge-pois a permis de réduire significativement le nombre de pucerons, comparé à l'orge seule ou à l'association orge-féverole. Toutefois en fin de saison aucune distinction entre les différentes associations culturales n'était observée. De même, l'arrangement spatial des associations culturales n'a pas montré de différences.

En ce qui concerne les insectes auxiliaires, coccinelles, syrphes et guêpes parasitoïdes ont été observées. Un plus grand nombre de ces auxiliaires était présent dans les cultures pures d'orge.

Sous serre, la transmission du PVY par *M.persicae* était étudiée dans des associations de pomme terre et orge. Dans un premier temps, les pucerons étaient préalablement infectés par le PVY. Puis avant d'être déposé sur une plante hôte, soit ils étaient déposés sur des feuilles d'orge, soit ne subissaient aucun traitement. Les observations des symptômes sur la plante hôte n'ont montré aucune différence entre l'association culturale ou la pomme de terre seule.

De même, une expérience menée en pots entre une association pomme de terre-orge ou une culture pure de pomme de terre, a montré que l'association culturale ne permet pas de réduire la transmission du PVY. En effet, que les pucerons soient préalablement infectés par le PVY ou qu'ils l'acquièrent directement sur un plant de pomme de terre infecté, le nombre de plante hôte présentant des symptômes de PVY était similaires entre les systèmes.

En conclusion de ce projet :

- Les associations culturales entre orge et pois ou orge et féverole présentent un intérêt dans la lutte contre le puceron des céréales *S. avenae*. Toutefois, leurs actions sur les insectes auxiliaires restent à démontrer.
- L'association pommes de terre et orge ne permet pas de réduire la transmission du PVY par *M. persicae*.

# Content

<b>ACKNOWLEDGEMENTS</b> .....	<b>6</b>
<b>RESUME DU PROJET</b> .....	<b>7</b>
<b>CONTENT</b> .....	<b>10</b>
<b>FIGURES LIST</b> .....	<b>12</b>
<b>INTRODUCTION</b> .....	<b>13</b>
<b>BACKGROUND</b> .....	<b>15</b>
WHAT IS INTERCROPPING? .....	15
WHY USE INTERCROPPING? .....	16
<i>Agronomic perspective: yield, nutrient and resources, benefits for the following crops.</i> .....	16
<i>Yield and production advantages</i> .....	16
<i>Light and water resources</i> .....	16
<i>Soil properties</i> .....	17
<i>Effect on the following year's crop</i> .....	17
<i>Ecological perspective: biodiversity</i> .....	17
<i>Economic perspective</i> .....	18
APHIDS .....	18
<i>Description</i> .....	18
<i>Life cycle</i> .....	18
<i>Feeding habits</i> .....	20
<i>Damage on crops caused by aphids</i> .....	20
<i>Aphids in the food web</i> .....	21
<i>How can aphids find their host?</i> .....	23
PEST CONTROL EFFECT OF INTERCROPS? .....	24
<b>PRESENTATION OF THE WORK</b> .....	<b>26</b>
<b>CHAPTER 1 : REDUCTION OF APHID NUMBER BY INTERCROP SYSTEMS</b> .....	<b>27</b>
INTRODUCTION.....	27
MATERIALS AND METHODS: .....	28
<i>Site.</i> .....	28
<i>Treatments.</i> .....	28
<i>Plant material</i> .....	29
<i>Aphid colonies</i> .....	29
<i>Counting</i> .....	30
<i>Statistical analysis</i> .....	30
RESULTS.....	31
<i>Observations from the yellow trap</i> .....	31
<i>Evolution of the S. avenae population.</i> .....	31
<i>Parameters influencing the number of aphids:</i> .....	32

DISCUSSION .....	36
<i>Evolution of the S. avenae population.</i> .....	36
<i>Parameters influencing the number of aphids:</i> .....	36
<i>Presence and number of beneficial insects.</i> .....	37
<i>Presence and number of beneficial insects.</i> .....	37
<i>Summary</i> .....	38
<b>CHAPTER 2 : VIRUS TRANSMISSION .....</b>	<b>39</b>
INTRODUCTION.....	39
MATERIALS AND METHODS: .....	40
<i>Experiment 1:</i> .....	40
<i>Experiment 2:</i> .....	42
RESULTS.....	46
<i>Experiment 1</i> .....	46
<i>Experiment 2</i> .....	47
DISCUSSION.....	49
<i>Experiment 1</i> .....	49
<i>Experiment 2</i> .....	50
<i>Why intercrops were not efficient in controlling virus transmission?</i> .....	51
<i>Summary</i> .....	51
<b>CONCLUSION .....</b>	<b>52</b>
<b>REFERENCES.....</b>	<b>53</b>
<b>APPENDIX .....</b>	<b>59</b>

# Figures List

FIGURE 1: REPRESENTATION OF THE DIFFERENT INTERCROPS STYLE: RELAY, MIXED AND STRIP OR ROW INTERCROPPING (BROOKER ET AL., 2015). ..... 15

FIGURE 2: REPRESENTATION OF APHID LIFE CYCLE ..... 19

FIGURE 3: REPRESENTATION OF AN APHID (MORPHOLOGY) AND ITS RELATIONS IN THE FOOD WEB (PREDATORS AND PARASITIDS ABOVE, PLANT BELOW). PICTURES OF THE PREDATORS AND PARASITIDS WERE TAKEN IN THE FIELD OF WOODLAND (SRUC- ABERDEEN CAMPUS)..... 23

FIGURE 4: EXPERIMENTAL DESIGN OF THE PLOTS IN THE FIELD, WITH ON THE BOTTOM LEFT A ZOOM OF ONE PLOT TO SEE AREA OF APHID INTRODUCTION AND COUNTING METHOD. .... 29

FIGURE 5: REPRESENTATION OF A PLASTIC BOX WHERE THE APHID COLONIES WERE KEPT BEFORE INTRODUCTION OF THE APHIDS IN THE EXPERIMENTS. .... 30

FIGURE 6: EVOLUTION IN TIME OF THE NUMBER OF *S. AVENAE* COUNTED IN THE FIELD PER TREATMENTS. .... 32

FIGURE 7: REPRESENTATION OF THE NUMBER OF *S. AVENAE*, A. BETWEEN THE DIFFERENT TREATMENTS, B. BETWEEN THE DIFFERENT PLANT SPECIES INTERCROPPED WITH BARLEY, AND C. IN RELATION WITH BENEFICIAL INSECTS NUMBER (WHEN PRESENT IN THE PLOTS) AND DEPENDING ON THE ARRANGEMENTS (NONE IN THE MONOCROP, ROW OR MIX IN THE INTERCROP). .... 34

FIGURE 8: REPRESENTATION OF THE STATISTICAL ANALYSIS. A. AVERAGE NUMBER OF *S. AVENAE* BETWEEN THE DIFFERENT TREATMENTS, B. NUMBER OF APHIDS IN RELATION WITH THE NUMBER OF BENEFICIAL BETWEEN THE DIFFERENT TREATMENTS, C. NUMBER OF APHIDS IN RELATION WITH THE NUMBER OF BENEFICIAL BETWEEN THE PLANT INTERCROPPED..... 35

FIGURE 9: EXPERIMENTAL DESIGN OF EXPERIMENT 1. TIMES REFER TO THE PERIOD FOR WHICH APHIDS STAYED IN THE COMPARTMENT. .... 41

FIGURE 10: DESIGN OF THE NETS USED TO KEEP THE APHID IN THE POT..... 41

FIGURE 11: EXPERIMENTAL DESIGN OF THE POTS FOR CONDITIONS 1 AND 2 ..... 44

FIGURE 12: POT DESIGN DURING THE EXPERIMENT..... 44

FIGURE 13: PICTURE SHOWING IN A. THE DIFFERENCES BETWEEN A HEALTHY *PHYSALIS* (ON THE LEFT) AND A PVY INFECTED ONE (ON THE RIGHT). IN B. IS A ZOOM OF AN INFECTED *PHYSALIS*. BOTH PICTURES ARE CONTRAST AND LIGHT MODIFIED TO INCREASE THE VISIBILITY OF THE SYMPTOMS. .... 45

FIGURE 14: RATE OF INFECTED *PHYSALIS* (I.E. NUMBER OF *PHYSALIS* PRESENTING PVY SYMPTOMS / TOTAL NUMBER OF *PHYSALIS* PER REPLICATE) IN EACH TREATMENT. .... 47

FIGURE 15: AVERAGE NUMBER OF *M. PERSICAE* PRESENT IN THE POTS. A. AND C. REPRESENT THE INCREASE OF THE POPULATION BETWEEN THE THREE COUNTS (WITH OR WITHOUT A POTATO PLANT IN THE POT RESPECTIVELY). B. AND D. REPRESENT THE NUMBER OF APHIDS THAT REACHED THE *PHYSALIS* (WITH OR WITHOUT A POTATO PLANT IN THE POT RESPECTIVELY). THE LETTERS INDICATE THE INFLUENCE OF THE TREATMENT IN THE MODEL, WHEN LETTERS ARE IDENTICAL THE TREATMENTS HAD NO INFLUENCE ON THE NUMBER OF APHID THAT REACHED THE *PHYSALIS*. .... 48

FIGURE 16: PREVALENCE OF PVY IN THE *PHYSALIS* PLANTS. A. REFERS TO THE RESULTS WHEN NO POTATO WAS PRESENT IN THE POT AND B. REFERS TO THE RESULTS WHEN A POTATO PLANT WAS IN THE POT. LETTERS ON THE GRAPHS REFER TO THE DIFFERENCES BETWEEN THE MODALITIES. WHEN LETTERS ARE THE SAME, THERE IS NO STATISTICAL DIFFERENCES. .... 49

# Introduction

Pest insects are known to cause a lot of damage to crop production (Ben Issa *et al.* 2016). The yield losses caused by herbivore pests are estimated to be 15% of the worldwide crop production (Maxmen 2013). Indeed, herbivores can reduce plant performances. When feeding on the flowers, they affect the whole fruit and seed production of the plant; when feeding on the leaves they alter growth, reproduction and vigour of the plants (Crawley 1989). Among herbivores, aphids are particularly damaging to the crops. One of the reasons can be their wide host plant range.

Aphids are the most important insect pest in Britain (Tatchell 1989; Ben Issa *et al.* 2016). They put the crops at risks because they are directly feeding on the plants (Savaris *et al.* 2013), thus affecting the yield or the quality (Tatchell 1989). They also damage the crops indirectly with plant virus transmission being a threat for farmers.

Insecticides are the most efficient method for controlling aphids (Tatchell 1989). However, resistance to some of the products (Farkas 2015; Ben Issa *et al.* 2016) and critics from the society pressurise for more sustainable pest management practices. As a result, interest in a heterogeneous agricultural environment for pest control has increased.

Moreover, cropping systems have evolved in most countries toward homogeneity and high input requirements (Matson *et al.* 1997; Anil *et al.* 1998; Tilman *et al.* 2002). However, it is known that plant diversity increases ecosystem services, among them is control of infestation from pests. Thus research has increased on diversity within agricultural environments (Anil *et al.* 1998). In some crops, it has been found that increasing genetic diversity can significantly lower yield losses due to pathogens (Matson *et al.* 1997) or to herbivores (Letourneau *et al.* 2011). Different strategies are used: planting hedges, agroforestry, stripes of flowers and mixtures are some of the examples.

Introducing plant heterogeneity to agricultural fields is carried out at different scales and might be used to control aphids. Some evidences show an influence of the landscape arrangement on the aphid abundance. The distance of 1,500m around a field has been determined as the most influential on the aphid populations (Carrière *et al.* 2014). At the field scale, diversity of plant species has an influence on aphid diversity depending on the creation of habitats. The density of aphid increases when plant diversity increases the number of habitats in the field. Whereas the reverse is observed in the case of a reduction of the habitat number (Bosem Baillod *et al.* 2017). In general, diversity in the field reduces the abundance of herbivores, and less damage is observed (Risch 1983; Letourneau *et al.* 2011; Dassou & Tixier 2016).

Plant diversity in the field can also act as a physical barrier to the aphids. Crop diversity may occur in time; it is referred to as crop rotation. Few studies looked at the effect of rotation on pest infestation. But it is likely that reducing the frequency of one crop in one field reduces infestations from its pests (the Colorado potato beetle being one example; Umaerus, 1992). In a system that increases plant diversity within one field, diverse plant characteristics might increase the difficulty for aphids to land on the crop. Another possibility is that the diversity camouflages the crop and the aphids have difficulty in finding the crop (Hooks & Fereres 2006).

Techniques such as “trap-crop” or “banker-plant” use knowledge about pest behaviour to protect crops. These plants (not always used as crops) are grown on the borders of the field of the crop that has to be protected. The trap-crop is a more attractive species in comparison to the crop (Hooks & Fereres 2006; Ben Issa *et al.* 2016). Whereas the banker plant is any plant that can disturb aphid behaviour and thus reduce damage in the crop, or attract natural enemies of the aphids (Frank 2010).

The results from studies using these methods to increase the diversity in the field are inconsistent. Admittedly, diversity brings arthropods (Dassou & Tixier 2016) and natural enemies of pests (Letourneau *et al.* 2011), but borders of a field are found to be less frequented by pests compared to its centre (Bosem Baillod *et al.* 2017).

Another way to bring plant diversity into a field is by associating different crops. Such systems are called intercrops, and are a way to cultivate two or more crop species in the same field and at the same time (Newton *et al.* 2009; Brooker *et al.* 2015).

The goal of this report was to review the knowledge that may help to understand the use of intercrops as pest and virus control and then design experiments to test this. Different cereal-legume intercrops and their impact on cereal aphids were investigated. Then the virus transmission from a potato aphid in a potato-barley intercrop was studied.

# Background

## What is intercropping?

One of the possible methods that can increase plant diversity within one field is to mix different crops. In the mixtures, the crop used can be of the same species, which is referred to as variety mixtures; otherwise the crops are from different plant species, which refers as species mixtures or intercropping. Intercropping is defined as the cultivation of two or more crop species at the same time within a field (Brooker *et al.* 2015).

The intercrops can be managed differently by altering crop species, time of sowing, sowing ratios or machinery used. Three major intercropping systems can be identifying (Ofori & Stern 1987; Brooker *et al.* 2015), they are represented Figure 1:

- Strip / row intercropping where the crops are grown at the same time in rows,
- Mixed intercropping where the crop species are grown at the same time with random sowing,
- Relay intercropping where one crop is sown before the other.

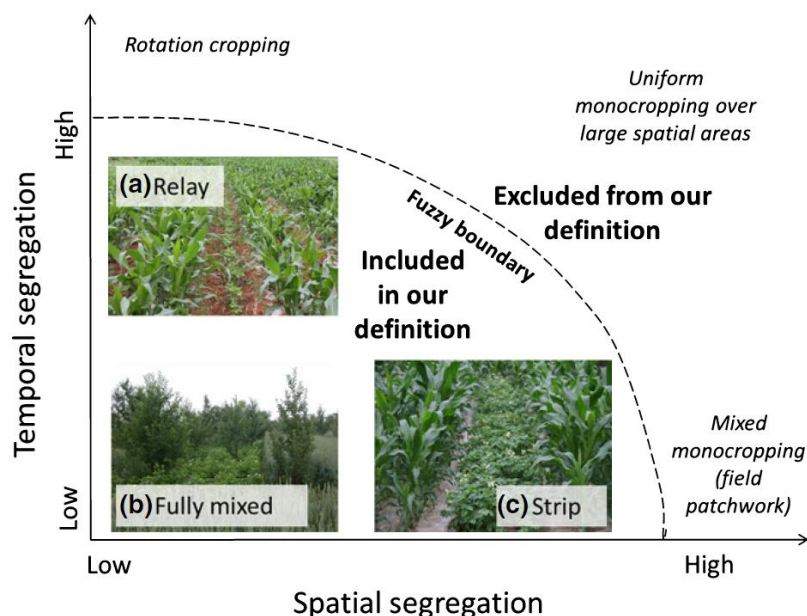


Figure 1: Representation of the different intercrops style: relay, mixed and strip or row intercropping (Brooker *et al.* 2015).

One of the most studied systems is intercrop between cereals and legumes. This interest is explained by the benefits from nitrogen fixation from the legume (Anil *et al.* 1998) and its



potential economic advantages (Vlachostergios *et al.* 2015). Indeed, this mixture is interesting for forage production as it has a better yield than the legume sole crop, and a higher crude protein content than the cereal sole crop (Lithourgidis *et al.* 2007; Vlachostergios *et al.* 2015). Little by little species mixtures are added to some agricultural systems or rotation. Especially in small scale farms running poly-culture and poly-livestock businesses, as intercrops can be grown for forage (Anil *et al.* 1998; Walker *et al.* 2009).

## Why use intercropping?

Agronomic perspective: yield, nutrient *and* resources, benefits for the following *crops*.

When two (or more) crops are growing together, competition for the resources might occur. As defined by Ofori and Stern (1987), competition between two plants growing together in the same field is over the search for the same resource, when its amount is below the combined need of the crops. Because species are different, intercrops are predicted to use different niches (Newton *et al.* 2009). This means that the needs and uptakes of the growth factors between the crops are slightly different.

In this regard, the choice of the crop species (Fujita *et al.* 1992; Lithourgidis *et al.* 2007; Hauggaard-Nielsen *et al.* 2008), the arrangement (e.g. random vs row mixtures, row spacing (Fujita *et al.* 1992)), the ratio of each species (Lithourgidis *et al.* 2007; Vlachostergios *et al.* 2015) and the sowing density (Ofori & Stern 1987) are important parameters to take into account, and may change the agronomy of the intercrop.

Yield and production advantages

Intercrops have shown agronomic potential when compared to pure stand crops; although results from intercrop production vary between studies (Fujita *et al.* 1992; Jahansooz *et al.* 2007; Newton *et al.* 2009; Yu *et al.* 2016). The Land-Equivalent Ratio (LER) is used to calculate and evaluate the efficiency of intercrops. It is the land area of sole crop that is needed to produce the same yield as the intercrop (Yu *et al.* 2016). It is shown that in average LER of cereal-legume intercrops is  $1.17 \pm 0.24$ , which indicates a better land use efficiency compared to the sole crops (Yu *et al.* 2016). Furthermore, the overall yield in intercrops is found to be more stable over years (Raseduzzaman & Jensen 2017). Such improvements can be explained by a better use of resources (Yu *et al.* 2016).

Light and water resources

In cereal-legume intercrop competition for light or water can occur. It is known that the light interception of one crop varies with its architecture (Ofori & Stern 1987). Cereals have long and narrow leaves compare to legumes that have short, wide ones. Thus in terms of light reception, each crop plant has a specific strategy. However, when one of the crops becomes taller than the other one, it produces shadow which results in competition for light. It has been observed in maize-bean intercrops the shadow from maize induced a reduced temperature in the intercrop (Castro *et al.* 1991).

For water uptake, intercrops are said to be more efficient (Jahansooz *et al.* 2007; Brooker *et al.* 2015). The water uptake depends on the characteristic of the plants; one can be more competitive than the other (Jahansooz *et al.* 2007). In the search of different plant characteristics, cereal-legume intercrops are interesting as their rooting system differ and as a result the water uptake is increased when compared to sole crop (Layek *et al.* 2018).

#### Soil properties

Intercrops can improve water use efficiency in comparison to a sole crop because of their impact on soil physical properties. Within these systems the soil organic matter is increased (Layek *et al.* 2018). This may directly improve the structure of the soil, as well as water retention and air circulation. Moreover, by adding a plant species, the biology of the soil might be different to the one found under a sole crop, with possible higher diversity. In addition the chemical properties of the soil are modified by intercrops with nutrients being a good example.

Nutrient uptake is different between cereals and legumes as legumes have the ability to fix atmospheric nitrogen (N). This is done in a symbiotic relationship with a bacterium, from the species *Rhizobium*, present in the plant roots. The symbiosis bacteria are located in specialised cells called nodules. As they access atmospheric N, legumes rely less on the soil N than cereals do (Justes *et al.* 2014) and the two crops are using different niches for N. As a result, cereal-legume intercrops have better N use efficiency (Ofori & Stern 1987; Fujita *et al.* 1992; Hauggaard-Nielsen *et al.* 2008). For instance, a 32% increase in N use has been determined in a wheat-pea intercrop (Bedoussac & Justes 2011). One explanation may be the N transfer from the legume to the soil (Fujita *et al.* 1992; Xiao *et al.* 2004; Hauggaard-Nielsen & Jensen 2005; Justes *et al.* 2014). Through root exudates or leaf decomposition on the soil, legumes release N that is available to the cereal (Fujita *et al.* 1992; Justes *et al.* 2014). This transfer of N between the crops is referred to as facilitation.

#### Effect on the following year's crop

Intercrops are shown to be beneficial to the following crop (Fujita *et al.* 1992; Walker *et al.* 2009), depending on the cereal-legume mixture (species and ratio).

It is considered as an advantage for the farmers because whole intercrop system has a better resource use efficiency compared to a single stand crop. Thus intercropping cereals and legumes could maintain good production and reduce the amount of input necessary.

#### Ecological perspective: biodiversity

As the environment quality is getting more consideration by the society, agriculture is closely monitored. The risks from chemical inputs are controlled, which leads to the prohibition of harmful products (either for the environment or for human health). Among the parameters taken into account for environment quality measurement, is biodiversity. To some extent the biodiversity has reduced in the current agricultural systems (Matson *et al.* 1997; Tilman *et al.* 2002; Letourneau *et al.* 2011). Suppressing certain species in an ecosystem can disturb the natural balance of ecological regulation, thus an increase of diversity may favour ecosystem

services. Using higher plant diversity in the fields may promote higher shelters and food supplies for organisms (beneficial ones being target in agriculture).

#### Economic perspective

As yield can be increase by intercrops, the economic benefit from this system is direct. Furthermore, higher protein content in cereals are observed when intercropped with legume (Lithourgidis *et al.* 2011; Bedoussac & Justes 2011; Vlachostergios *et al.* 2015), it increases the grain quality and so the marketable opportunities for it. Then, because facilitation may occur within the system, intercrops between cereals and legumes can be used in low input systems (Bedoussac & Justes 2011). Despite the lower yield of the intercrop in comparison to the sole crop, the overall economy of intercrops might be advantageous for farmers (Fujita *et al.* 1992). For instance, red pea-cereal intercrops have shown a monetary advantage index compared to the sole crop (Vlachostergios *et al.* 2015).

## Aphids

#### Description

Aphids are Hemipterans from the Aphidoidea superfamily. In terms of morphology (Figure 3) aphids comprise: head (with a pair of antennae, eyes, and rostrum), thorax (with three pairs of legs and sometimes wings) and an abdomen (usually with a pair of cornicles and a cauda). Their morphology varies between their different life stages and according to their living conditions. Indeed, aphids undergo four nymphal stages and an adult one. Moreover, apterous aphids are produced when the conditions are good; whereas winged individuals are produced when the conditions worsen (Kennedy & Stroyan 1959; Dixon 1985).

#### Life cycle

Aphids produce both parthenogenetic and sexual individuals depending on climate and growth stage (Dixon 1985). There are many different species in the Aphididae family; they may have different life cycles. The most common one is explained Figure 2 below (monoecic holocyclic cycle).

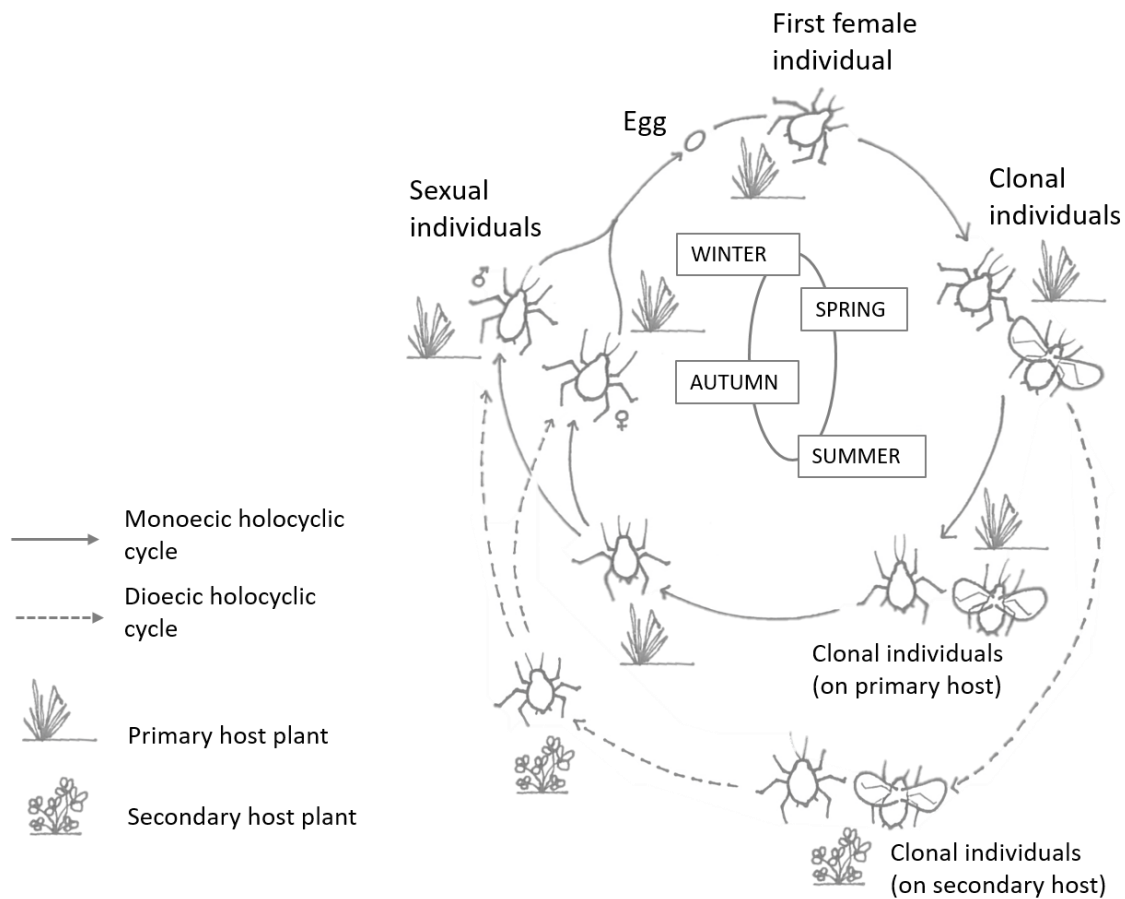


Figure 2: Representation of aphid life cycle

The sexual individuals lay eggs during autumn or winter (Dixon 1985; Fraval 1998a; Turpeau *et al.* 2011, 2018d). At this stage temperatures are important for the future development of the aphids. Indeed, a cold period to enter into diapause is necessary for their later growth (Dixon 1985) and to determine their flying behaviour (Turpeau *et al.* 2011).

The eggs hatch at the end of winter around host plant budburst (Dixon 1985; Turpeau *et al.* 2011, 2018d). The individuals produced are females, either apterous or alate depending on the environmental conditions (Turpeau *et al.* 2011, 2018d). Using viviparous parthenogenesis, these females produce live offspring that will become apterous females (Fraval 1998a). That is the start of a new colony.

Once settles on its host plant the aphid population grows and the clonal individuals are produced through parthenogenesis, especially during spring and summer. Environmental conditions determine whether apterous or alate aphids develop. Plant physiology (such as senescence) or aphid's population density ("group effect") induce the production of alates (Dixon 1985).

Early summer is characterised as the flying period for aphids. They spread, and look for new hosts to colonise (Turpeau *et al.* 2011, 2018d). When days shorten and temperatures reduces sexual individuals are generated, then the winter egg is produced.

The life cycle differs depending on the aphid species. For example *S. avenae* (the grain aphid) has a monoecic holocyclic cycle, which refers to a life cycle reproduced on the same plant

species throughout the year. Whereas, *R. padi* (bird cherry oat aphid) and *M. dirhodum* (rose grain aphid) have a dioecic holocyclic cycle, which refers to a life cycle that occurs on two different host species. The winter egg is laid on one plant species, then after a few generations alate individuals are produced and leave the first plant species to another one. New generations develop and before winter alate individuals are produced and fly again to the winter host plant species (Fraval 1998a; Turpeau *et al.* 2018d).

#### Feeding habits

Aphids are herbivorous (Figure 3), they are pricker-sucker insects and feed on their host plant (Kennedy & Stroyan 1959; Dixon 1985). They insert their rostrum in the plant cell to reach the phloem and extract nutrients they need from the sap with their stylet (Kennedy & Stroyan 1959; Turpeau *et al.* 2018d). Young leaves produced in spring are generally preferred by the aphids because of the amino acid content of these parts (Kennedy & Stroyan 1959; Dixon 1985). Thus young plants are more susceptible to aphid attack compared to more mature ones (Carrière *et al.* 2014).

#### Damage on crops caused by aphids

When aphids are on a plant they can cause different types of damage:

##### Direct damage

Aphids weaken the whole plant by feeding directly on parts of the plant. When it comes to agriculture, food production can be threatened; as yields can be reduced. The extent of this damage depends on the number of aphids and the time they are feeding on the plant (Tatchell 1989; Turpeau *et al.* 2018e). In cereals, direct damage can occur during the growth period and be up to 1% decrease in grain weight/plant (for 100 aphids feeding in one day) (Savaris *et al.* 2013). Damage by aphids also resulted in a 2.5t/ha decrease in the final grain yield (Turpeau *et al.* 2011). Aphids release saliva when feeding on plant material which may cause a reaction from the plant and ultimately alter its development (Turpeau *et al.* 2018e).

##### Indirect damage

Indirect effects are also recorded. Aphids extract the nitrogenous matter from the sap, in return they excrete the carbohydrates they don't use, so called the honeydew emissions (Kennedy & Stroyan 1959; Turpeau *et al.* 2018e). The honeydew is ejected onto the plant itself producing sticky surfaces, especially when the number of aphid is high. It can cause indirect damages to the plant because it alters the photosynthesis efficiency, especially by reducing light interception (Rabbinge *et al.* 1981). Furthermore, honeydew can be the substrate for fungus development, such as black mould (Turpeau *et al.* 2018e), which enhances damage to the crop.

In addition, aphids are virus vectors. They can carry and transfer virus from one plant to the other. The damage following a virus transmission by aphids is often more severe than damage caused by direct feeding. For instance, in one experiment 6% of the damage observed on cereals could be explained by direct feeding while 25% was explained by virus transmission (Valenzuela & Hoffmann 2015). The type of virus aphids transmitted and the efficiency of the transmission is dependent on aphid behaviour (such as the duration of the probing period or the effect of fasting: Day and Irzykiewicz, 1954).

## Aphids in the food web

In the food web, aphids are predated or parasitized by other animals and insects. Indeed, herbivores are part of a community with three trophic levels. The herbivores are feeding on a plant and natural enemies (predators, parasitoids or parasites) are feeding on the herbivores (Price *et al.* 1980). Among natural enemies of aphids there are different types. Some are generalists and while aphids are part of their diet, they also rely on other food resources for their survival. Others are specialists, in this case aphids are their only food resources; they are aphidiphagous. There are two levels of speciality as either the natural enemy can eat different aphid species or it only feed on one aphid species.

Some of the major natural enemies of aphids are presented below.

### Predators.

One well known aphid predator is *Coccinella septempunctata* (Coleoptera, Coccinellidae). *C. septempunctata* undergoes three development stages: egg, larva and pupa (Beverley 2010). One individual can lay about 200 to 500 eggs in its life time. The time duration of larval and pupal stage is highly dependent on their prey type (Hagen & van den Bosch 1968; Özder & Sağlam 2003; Beverley 2010).

Larvae (Figure 3) and adults individuals feed on aphids, other arthropods and pollen (Beverley 2010). The aphid species they feed on is not specific but it influences their development, fecundity and mortality (Özder & Sağlam 2003; Beverley 2010). Generally larvae feed from the body fluids of the aphids, whereas the adults eat the whole insect (Beverley 2010).

Among cereal aphids, it has been found that *S. avenae* is a good prey for *C. septempunctata*. Indeed it has a short developmental period and a low fecundity when feeding on this species. *R. padi* was found to be a poor prey (Özder & Sağlam 2003).

The most important aphid predators are larvae from the Syrphidae (Diptera) family (Hagen & van den Bosch 1968). Three developmental stages (egg, larva and pupa) are necessary for the syrphids to become adult (UC-IPM 2014). Syrphid eggs are laid close to aphids and in abundant populations (Hagen & van den Bosch 1968; Turpeau *et al.* 2018a). Between 500 and 1000 eggs are laid by one syrphid female (Turpeau *et al.* 2018a). The larvae (Figure 3) are aphidiphagous for some species (Turpeau *et al.* 2018a). They thus feed on aphids but their specificity for one particular aphid species varies. They can eat between 400 and 800 aphids during their development. The duration of the larval stage depends on the environment conditions. The larvae suck the body fluid of the aphids (Turpeau *et al.* 2018a). The adults feed on nectar, pollen and honeydew (Hagen & van den Bosch 1968; Turpeau *et al.* 2018a).

Other arthropod species, for example Chrysopidae (Neuroptera) and Hemerobiidae (Neuroptera), also are predators of aphids (Hagen & van den Bosch 1968; Turpeau *et al.* 2018b). Generally both the larvae and the adult feed on them, however larvae is much voracious than adults. For instance, the larvae can feed on 200 to 500 aphids during their development (Hagen & van den Bosch 1968; Turpeau *et al.* 2018b).

### Parasitoids.

Parasitoids kill their host as they develop within them (Figure 3), the host being the food supply of the larvae (Turpeau *et al.* 2018c). Most of the parasitoids of aphids are Hymenopteran from

either Aphelinidae family or Aphidiinae sub-family (Hagen & van den Bosch 1968; Turpeau *et al.* 2018c). The parasitoids wasps are solitary endoparasites of aphids (Hagen & van den Bosch 1968; Turpeau *et al.* 2018c). They are highly sensitive to temperature which can impact on their fecundity, development time and longevity (Hagen & van den Bosch 1968).

Individuals from the Aphidiinae sub-family have a narrow range of aphid specificity (Hagen & van den Bosch 1968; Turpeau *et al.* 2018c). They are attracted by their host's odour, especially honeydew (Hagen & van den Bosch 1968), and recognise them after antennae contact (Hagen & van den Bosch 1968). They insert their ovipositor bringing forward their abdomen between the legs (Turpeau *et al.* 2018c). The number of eggs deposited depends highly on the species, the temperatures and the environmental conditions (Hagen & van den Bosch 1968). Only one egg per aphid is deposited and all aphid stages can be parasited. When the larvae develops in the aphid's abdomen the aphid is being transformed to a mummy (inflated aphid covered by silk) (Hagen & van den Bosch 1968; Turpeau *et al.* 2018c). The emergent wasps are mainly females, and are able to oviposit just after they emerge. Adult wasps feed on honeydew or don't feed (Hagen & van den Bosch 1968).

In the Aphelinidae family a few species attack aphids. Not all the species palpate the aphid with its antennae, but when an aphid is chosen, the wasp turn its body to insert its ovipositor in the insect (Hagen & van den Bosch 1968; Turpeau *et al.* 2018c). The larvae stages are preferred for the oviposition and one egg is generally deposited (Hagen & van den Bosch 1968). The aphid mummy become black while the larvae develops. The emergent females have few eggs, but the ovogenesis is continuous in these individuals. Adults feed on aphid's body fluids mainly (Hagen & van den Bosch 1968; Turpeau *et al.* 2018c).

The action of natural enemies in the field can be of great interest for farmer to control pests. Some of the natural enemies presented here are used for biological control (i.e. use of natural mechanisms and interactions to control pests, especially through the introduction of macro-organisms, micro-organisms, chemical mediators or natural substances), and are commercially available for farmers. They have been particularly successful in enclosed environments such as greenhouses.

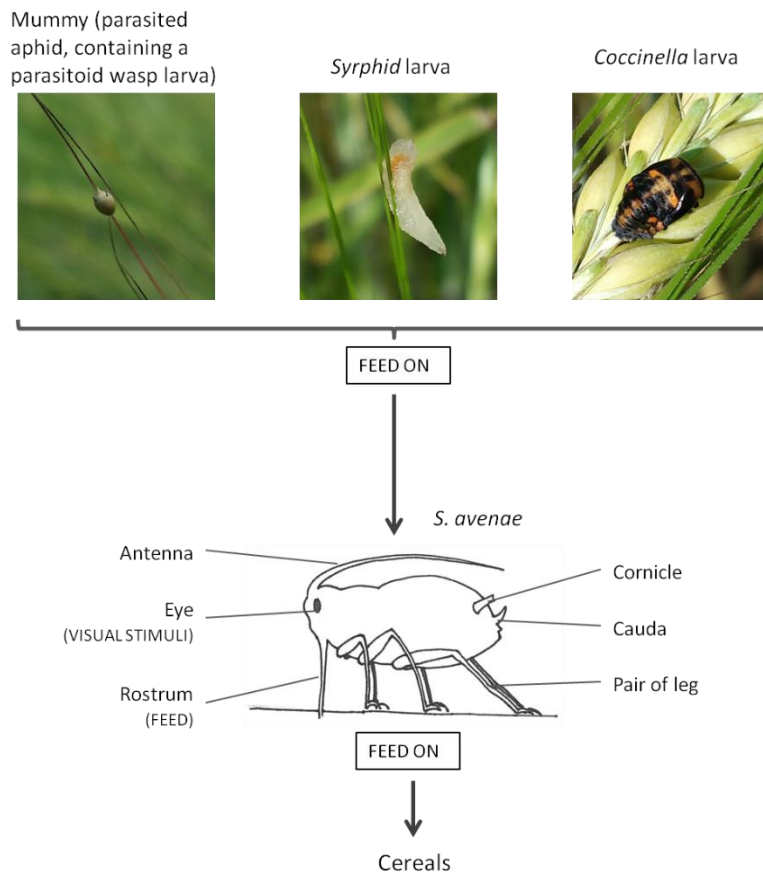


Figure 3: Representation of an aphid (morphology) and its relations in the food web (predators and parasitoids above, plant below). Pictures of the predators and parasitoids were taken in the field of Woodland (SRUC-Aberdeen campus).

How can aphids find their host?

Aphids have to find their host to grow and build colonies. Depending on the species the new individuals change host during spring. In other situations migratory aphids leave their plant to find a new host. Both types, alate and apterous, are able to disperse on relatively long distances, one flying and the other one walking (Ben-Ari *et al.* 2015). However, when the living conditions are getting more difficult, alate aphids develop and spread in the ecosystem. Alate aphids are better individual for spread as they use wind currents to fly, thus can reach more distant locations (Schröder *et al.* 2017). During this dispersion process the aphids are looking for a new host plant.

The aphid host finding behaviour can be divided into distinct steps (Powell *et al.* 2006; Döring 2014):

- The plant finding process, either walking or flying, the aphids need to find their host plant to survive. Sensory stimuli are used (Powell *et al.* 2006; Schröder *et al.* 2017). For aphids it has been shown that colour has an attractive effect, especially yellow (Powell *et al.* 2006; Döring 2014). Further studies found that not only the colour will influence the landing of an aphid but also the contrast between the plant and its background (Hooks & Fereres 2006; Döring & Röhrig 2016). The greater the contrast, the greater the chance of landing.



- The landing (for flying individuals): it is a decision (Döring 2014) that the aphid makes when the different stimuli attract it to one plant. The main attractant for the aphids to alight on a plant is the plant volatiles (Powell *et al.* 2006). Indeed, aphids seem to be able to recognise either single or mixture compound(s) from their host plants, however they are repelled by volatiles emitted from non-host plants (Schröder *et al.* 2017).
- The probing: the aphid inserts briefly its stylet into the parenchyma cells of the plant. As host finding is a matter of chance (Döring 2014), aphids need to probe the plant they landed on, to determine whether it is their host (Powell *et al.* 2006; Hooks & Fereres 2006). The first probe occurs after a general acceptance of the plant surface characteristics. It would be a brief probe that just goes to the epidermis (Pirone & Perry 2002; Powell *et al.* 2006). If the sap is recognised, longer probes will occur for better discrimination. The probing is repeated as long as a host plant is found, but also depending on the settlement strategy of the aphids (Döring 2014; Schröder *et al.* 2017).
- The phloem acceptance. Finally when the sap is fully recognised, aphids settle for sap ingestion (Powell *et al.* 2006).

## **Pest control effect of intercrops?**

Aphids are sensitive to odours and colours to find their host plants (Powell *et al.* 2006; Döring 2014; Schröder *et al.* 2017). It is therefore likely that higher diversity in an environment increase the difficulty for aphids to find their hosts. This phenomenon has been observed at different scales.

Furthermore, either decrease or increase of pest numbers have been observed in intercrops compared to pure stands. Some examples showed that at the maximum growth of the colony, lower density of aphids were found in the intercrop (Lopes *et al.* 2015; Lai *et al.* 2017). In other cases, intercrop showed an increased number of aphids (Helenius 1989). The result appears to depend on the type of intercrop studied. The proportions of each plant species, the arrangement, and the row space may change the overall influence of the intercrop to control the pest (Letourneau *et al.* 2011).

There are different theories to explain these results. They involve either pest or natural enemy behaviour.

On the one hand is the “*natural enemy*” hypothesis, first described by Root (1973). It states that the natural enemies of herbivores will be more effective when there is diversity in the environment (Helenius 1989). Indeed, these beneficial insects will be able to find a greater diversity in food supply, such as more herbivore species with various occurrence times, but also more nectar or pollen. Diversity will also bring more habitats, because of different plant architecture (Trenbath 1993). Various habitats and diversity of food type may attract other insects, some of them being predators or parasitoids of the pests. Thus the possible controlling effect from an intercrop may come from a higher number of natural enemies that regulate the aphid populations. This system would appear to favour the generalist aphid predators where adults have a wider diet, including plant products.

On the other hand is the “*resource concentration*” hypothesis. It was also suggested by Root (1973), where he described the hypothesis as a “trapping effect” on herbivores. Herbivores and insects in general, tend to be attracted by area where their host is in high number (concentrated). Thus, herbivores are expected to be more effective on a pure stand where their host plant is in great quantity (Helenius 1989). However, more diverse environment, such as intercrops, will confuse the insects in their search of hosts (Lopes *et al.* 2015). It can be related to lesser number of the host, or visual / olfactory disturbances (Trenbath 1993; Hooks & Fereres 2006). In this case aphids are less likely to land on intercrops (compared to a pure stand field).

Finally, intercrops have also been studied with regard to viruses. The use of intercrop to the reduce transmission spread from aphids has been debated. The theory behind it is the “*virus sink hypothesis*” (Irwin *et al.* 2000). It is related to non-persistently transmitted virus. When aphids are looking for their host plant they probes on the plant they land on. It is expected that after few probes on non-host plant the aphids will lose the virus and reduce their infectivity (Irwin *et al.* 2000; Hooks & Fereres 2006). Plus, the presence of another species in the field may increase the difficulty for the aphids to find their host (Irwin *et al.* 2000). Thus it is expected that the intercrop will disrupt the efficiency of virus transmission.

# Presentation of the work

In cereals various aphid species can visit the crops, but there are three main species commonly found in the field in Scotland. They are *Metopolophium dirhodum*, *Sitobion avenae* and *Rhopalosiphum padi* (sasa and ephytia). They damage cereal crops by feeding on the crop, and by spreading Barley Yellow Dwarf Virus (BYDV). Another threat is the transmission of the Potato Virus Y (PVY) to potato crops, as these three species are vectors of this potato virus (SASA 2018a). It is important to control the aphid population, and especially their dispersion to potato field because the PVY is the most harmful virus in potatoes (Valkonen 2007). Despite the fact that these aphids are not hosted by potato, they can transmit the virus while landing on a potato plant (Valkonen 2007).

Furthermore, potato plants can be used as hosts by six main aphid species. The most numerous in Scotland is *Macrosiphum euphorbiae*, however *Myzus persicae* is the most important in most of the European countries (SASA 2018b). *Myzus persicae* is of particular interest because it a good virus vector particularly for PVY (SASA 2018c).

The aim of the study was to determine whether intercrop systems could act as an aphid control in terms of development of their population and virus transmission. Different mechanisms by which intercrops can help to control these pests were tested, and divided in two chapters. In the first chapter the development and dispersion of the cereal aphids in a cereal legume intercrop are considered. The objectives were:

1. To determine whether the presence of a non-host plant in the main crop could act as a physical barrier to the aphid dispersal.
2. To evaluate the efficiency to control cereal aphids of different intercrops based on crop species and arrangement.
3. To assess the role of the natural enemies in intercrop compared to pure stand of barley.

The second chapter is about non-persistent virus transmission in intercrops. The intercrop system used was barley-potato, and the non-persistent virus studied was PVY. The main objective was to determine whether the introduction of a non-host plant can affect virus transmission of PVY.

# Chapter 1 : Reduction of aphid number by intercrop systems

## Introduction

*S. avenae* is one of the cereal aphids. It is found in high numbers in Scotland (SASA 2018a) and develops mainly on spring cereals (as well as *M. dirhodum* and *R. padi* the other cereal aphids). Early season they attack the leaves and when the ears appear they migrate to develop on them. They can reduce the number or quality of the grain when their population is numerous.

Intercrops are studied for the numerous benefits they are expected to provide to crops. However, their ability to control pests is questioned. Indeed, by increasing the number of plant species the number of arthropods is expected to increase as well (Helenius 1989; Xie *et al.* 2012; Liu *et al.* 2017). However it is difficult to predict whether it will increase pest insects (Root 1973; Helenius 1989) or beneficial insects (Root 1973; Trenbath 1993; Xie *et al.* 2012; Liu *et al.* 2017).

Theories, such as the “*natural enemy*” and the “*resource concentration*” hypotheses, help to understand the possible mechanisms that occur in intercrops. However results from different studies vary, and support either both or neither hypotheses. In some cases a higher number of aphids was found in pure crop plots, compare to the intercrops (Trenbath 1993; Lopes *et al.* 2015, 2016; Lai *et al.* 2017). These support the “*resource concentration*” hypothesis. Other cases found that the intercrop increase the number of alate aphids, compared to the monocrop (Helenius 1989, 1991).

Differences found on the effects of intercrops on aphid populations can be explain by space and time parameters. Indeed, the arrangement of the intercrops provides different aphid phenology. Randomly mixed intercrops seem to have a better controlling effect (i.e. reducing the number of aphids) compare to row intercropping (Lopes *et al.* 2015). The proportion of each crop has also an impact on the aphid population. For example in a wheat-maize intercrop, the less wheat in the ratio the higher the number of cereal aphids were found (Liu *et al.* 2017). The crop species and the sowing density used may also affect the efficiency of intercrops to act as a pest control (Singh *et al.* 2017).

Furthermore, variations in time have been observed. The number of aphids between an intercrop and a sole crop varies depending on the growth stage of the aphid population. The aphid colonisation seems to be delayed in the pure stand, with higher number in the intercrop

at that stage. Later the pattern of colony growth is reversed and higher numbers of aphids are found in the pure stand compared to the intercrop (Liu *et al.* 2017).

The aim of the current study was to determine whether intercropping has a controlling effect on the aphid population. The following hypotheses were tested:

- i) An intercrop between cereal and legume would reduce the number of cereal aphids.
- ii) The arrangement of the intercrop changes the spread of aphids, with mixed intercropping providing more efficient control.
- iii) The legume species used changes the dispersion of the cereal aphids, with more dispersion when barley is intercropped with peas compared to beans, as the peas may act as a bridge between the barley plants.
- iv) Natural enemies might be more abundant in the intercrops.

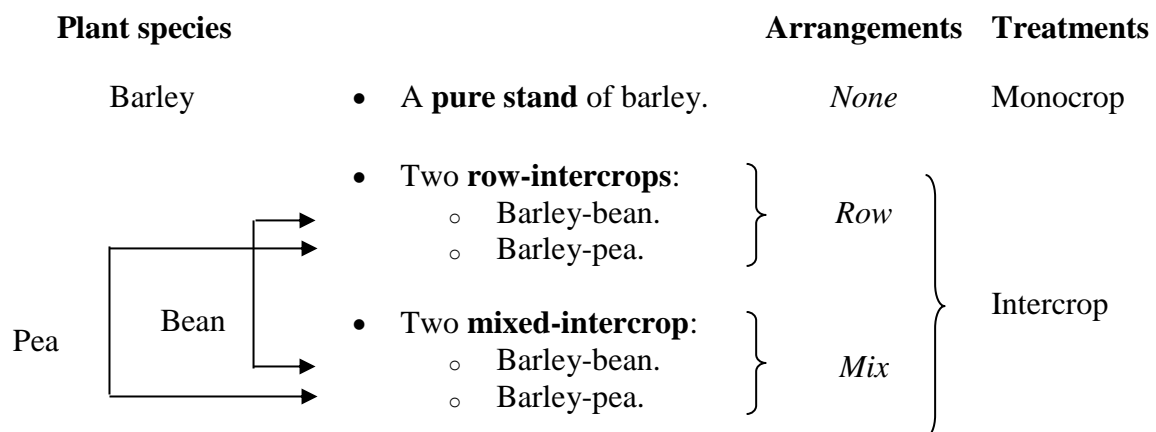
## Materials and Methods:

Site.

All the experiment were conducted at the Scotland’s Rural College (SRUC) Aberdeen campus (Craibstone Estate, Aberdeen, AB21 9Y1). The field experiment was located next to the research building, in the “Woodland” area. The Woodland soil is a sandy loam, and partly sheltered by trees.

Treatments.

The aim of this field experiment was to determine the effect of intercropping on the development of aphid colonies. Thus, five different designs were used and arranged in a latin-square (see Figure 4); they comprised different arrangements (row or mix) and plant species (bean or pea) in the intercrop systems with barley.



No pure stands of bean or pea had been sown because only the development of cereal aphids toward barley was recorded. The cereal aphids do not colonise legumes. The different species intercropped were used to compare the influence of their structures on the spread of aphids. The different arrangements were established to evaluate their influence on aphid dispersion.

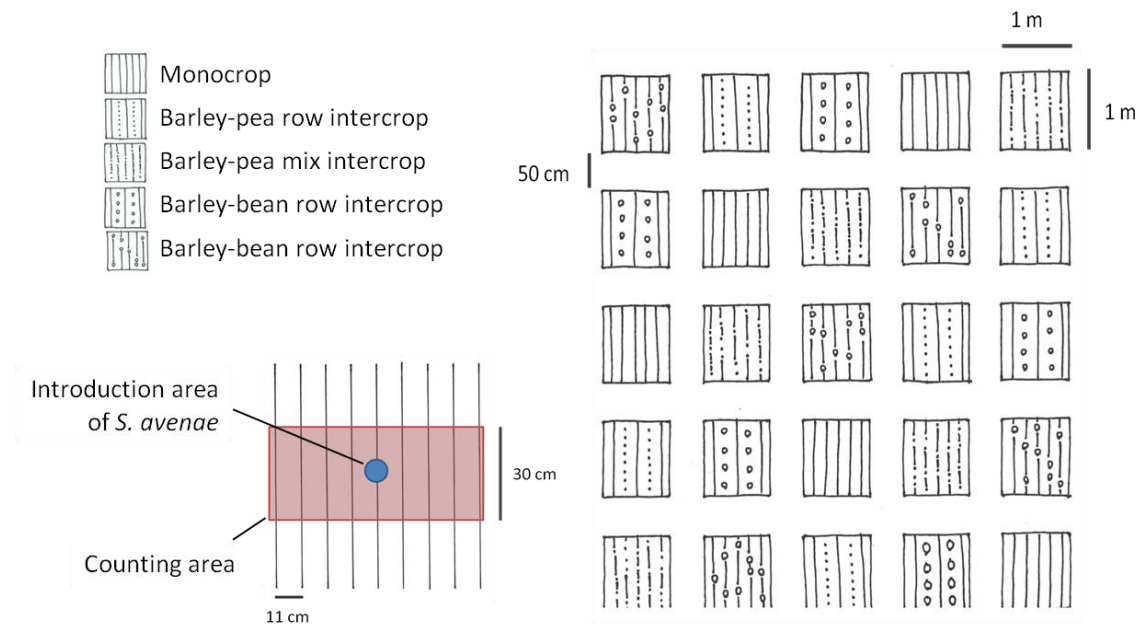


Figure 4: Experimental design of the plots in the field, with on the bottom left a zoom of one plot to see area of aphid introduction and counting method.

#### Plant material.

On the 1<sup>st</sup> of May 2018 all the seeds were hand sown in the Woodland field. The barley seeds used had been grown organically and were Westminster variety. The bean seeds were also grown organically of the Fanfare variety and the pea seeds were fungicide treated, from the 04 variety. The barley was sown about 2-3 cm deep in the ground at the rate of 200 kg/ha. Bean and pea were sown deeper, about 5-7cm. The intercrops (replacement type) used 60% barley and 40% legumes. Bean sowing rate was 50 seed/m<sup>2</sup> and pea sowing rate was 100 seed/m<sup>2</sup>. The plot area for this experiment was 1m x 1m, and the space between the rows was 11-12 cm, which is equivalent to 9 rows in each plot.

In the row intercrop the middle row was always sown with barley, from that the legumes rows alternated outward. This led to 5 rows of barley and 4 rows of one legume (either bean or pea depending on the plot). In the mixed intercrop the barley and legume were randomly sown, which led to mixture of barley and one legume (either bean or pea depending on the plot) in the same row.

#### Aphid colonies.

The cereal aphid, *Sitobion avenae*, was used in this experiment. 30 individual per plots were added the 6<sup>th</sup> June, to insure its presence in the field. They were provided by the James Hutton Institute (courtesy of Dr Gaynor Malloch). They were kept in double compartment plastic pots (see Figure 5) until the day of release in the field. In these plastic boxes, aphids were located in the biggest compartment, and were fed with barley leaves, in order to keep the colonies

growing. The barley had access to the second compartment which was filled with water to maintain the leaves.

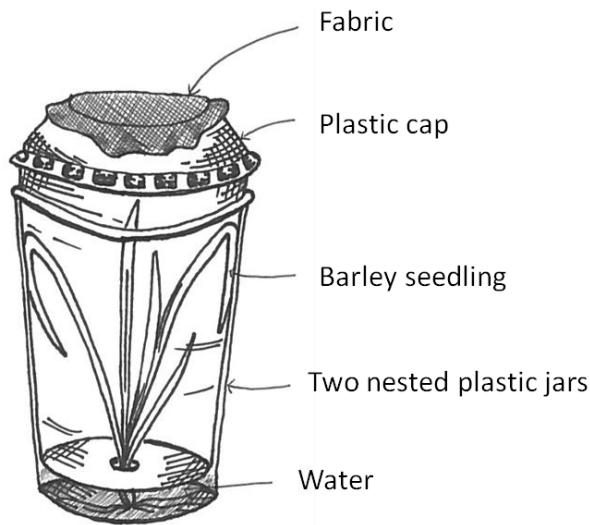


Figure 5: Representation of a plastic box where the aphid colonies were kept before introduction of the aphids in the experiments.

#### Counting.

*S. avenae* were counted twice a week and then once a week for the three last counting. A distinction between adults, alate aphids or nymphs was made while counting the aphids. The differences were observed and based on the presence of wings (alate aphids) or not and based on the size of the individuals ( $< 1$  mm for nymphs and  $> 2$  mm for adults). In addition, beneficial insects were recorded. Visual observation of mummies and larvae indicated that the natural enemies were parasitoid wasps, ladybirds and syrphids (hoverflies). Their number were recorded, the term “beneficial” is used to describe them.

A yellow trap (Ringot Nickerson model) was put in the middle of the field. The yellow trap was filled with 800ml water containing one drop of detergent. It was implemented for one week, and then the insects were collected and put in 70% alcohol solution before classification by insect orders. The yellow trap was used three times during the experiment.

#### Statistical analysis.

The number of *S. avenae* counted on barley plants was the primary variable examined in the analysis (data of *S. avenae* on the legumes was not used as only a few aphids were counted on these plant species).

A first general analyse was made to determine the influence of treatments and date of counting. The number of aphids showed a Poisson distribution. The use of a generalised linear model was appropriate (family: Poisson, link: logit).

The data was then separated into two different periods: the “development of the aphid colonies” (development) and the “peak of the colonies” (peak). The 9<sup>th</sup> July was the date when higher number of aphids was counted in the field, and was used as the date to separate the two periods.

Number of aphids was recorded at different date; to take into account this repeated measurements in time, the plots were used as a random factor. This includes the variation that exists naturally between the plots in the model. Intercrops parameters and natural enemies were analysed. The model used is shown below:

$$\text{Number of } S. \text{avenae} \sim \text{Treatments} * \text{Arrangement} * \text{Plants} \\ * \text{Number of beneficial} + (1 | \text{Plots})$$

*Treatment* is either monocrop or intercrops. *Arrangement* states for none, row or mix. *Plants* refers to either barley, bean or pea.

The main difference between the analyses of the two time period (i.e. development or peak of the colonies) was the distribution of the number of aphids. It followed a Poisson distribution during the development of the colonies (use of a generalised mixed linear model) whereas it was normal at the peak of the colonies (use of a linear mixed model).

The model was extended using the “lme4” package from the statistical software R version 3.3 .2 (2016-10-31) by using the function “glmer” for generalised mixed linear model, and “lmer” for mixed linear model. The difference between the parameters of the models was tested by an analysis of the variance method: ANOVA (type III).

## Results

Observations from the yellow trap.

In the experiments an initial infestation was inoculated into the plots. This was done at a time prior to natural colonisation and ensured uniformity of infestation and replication. However the open field also experienced external landing of aphids which was detected by the yellow water trap. 3, 9 and 77 aphids were monitored by yellow traps, 12<sup>th</sup> June, 2<sup>nd</sup> July and 18<sup>th</sup> July respectively. By the third sampling, the highest number of aphids was recorded. The species of aphids in the trap were not identified, but it is likely they were from tree colonising species as the field was surrounded by trees and not agricultural land.

Evolution of the *S. avenae* population.

No delay in the expansion of the colonies was observed between the different treatments, Figure 6. The development of the colonies occurred in June until flowering of the barley (approx. 5<sup>th</sup> July) when the growth peak was observed. Evolution of the location of aphids is presented in the appendix. The colonies maintained higher number of individuals until the beginning of August (approx.. 2<sup>nd</sup> August).

The statistical analysis on the total number of aphids showed that the treatment, the date of counting and their interaction had a significant influence (p value =  $7.26 \times 10^{-4}$ ,  $\ll 0.05$  and  $\ll 0.05$  respectively). For a better understanding of the results, the following analysis were separated between the period in which aphid colonies developed or peaked.



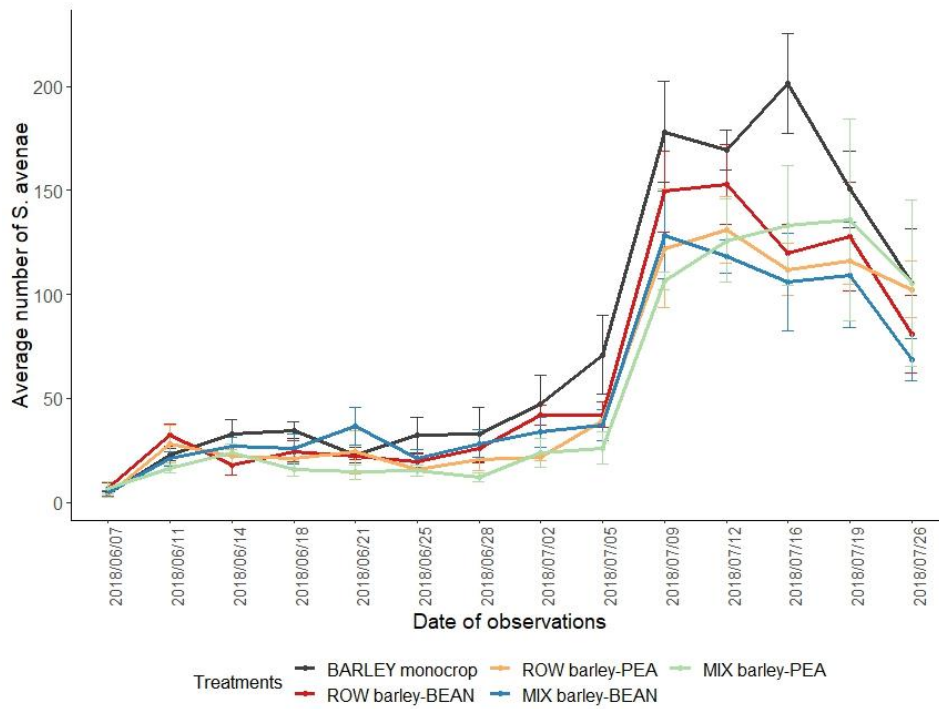


Figure 6: Evolution in time of the number of *S. avenae* counted in the field per treatments.

Parameters influencing the number of aphids:

Results of the statistical analysis are presented in Table 1.

Table 1 : Result of the statistical analysis done on the number of *S. avenae* counted in the field. The numbers in bold refer to significant parameters in the model.

Parameters	<i>P_value</i>	
	Development of the colonies	Peak of the colonies
<i>Treatment</i>	<b>0.00099</b>	<b>0.0076</b>
<i>Plant</i>	<b>0.018</b>	0.33
<i>Arrangement</i>	0.15	0.14
<i>Number of beneficial</i>	0.28	<b>0.0025</b>
<i>Plant : Arrangement</i>	0.20	0.54
<i>Treatment : Number of beneficial</i>	0.51	<b>0.017</b>
<i>Plant : Number of beneficial</i>	0.40	<b>0.019</b>
<i>Arrangement : Number of beneficial</i>	<b>0.040</b>	<b>0.033</b>
<i>Arrangement : Plant : Number of beneficial</i>	0.92	0.061

### *Development of the colonies:*

#### *Intercrop system.*

Number of aphids were significantly different between the treatments (Table 1 p\_value = 0.00099). The monocrop treatment had a significant higher number of aphids compared to the intercrops (see Figure 7 a.). The average number of aphids in the monocrop was  $33 \pm 4$  whereas it was  $23 \pm 1$  in the intercrop.

Differences in number of *S. avenae* were not significant between the three arrangements plant species (Table 1, p\_value = 0.15). The plants had a significant influence on the number of aphids (p\_value = 0.018). Barley and bean had similar abundance in aphids, but were significantly different from the pea. The association barley-pea showed lower number of *S. avenae* (Figure 7 b.). However, the interaction plant : arrangement had even number of *S. avenae* (Table 1, p\_value = 0.20).

#### *Number of beneficial insects.*

Number of beneficial had no significant influence on the number of aphids in the field (Table 1, p\_value = 0.28).

Interaction arrangement : number of beneficial was significantly different (Table 1, p\_value = 0.040). Figure 7 c. shows that the row intercrops contain both higher numbers of beneficial insects and aphids.

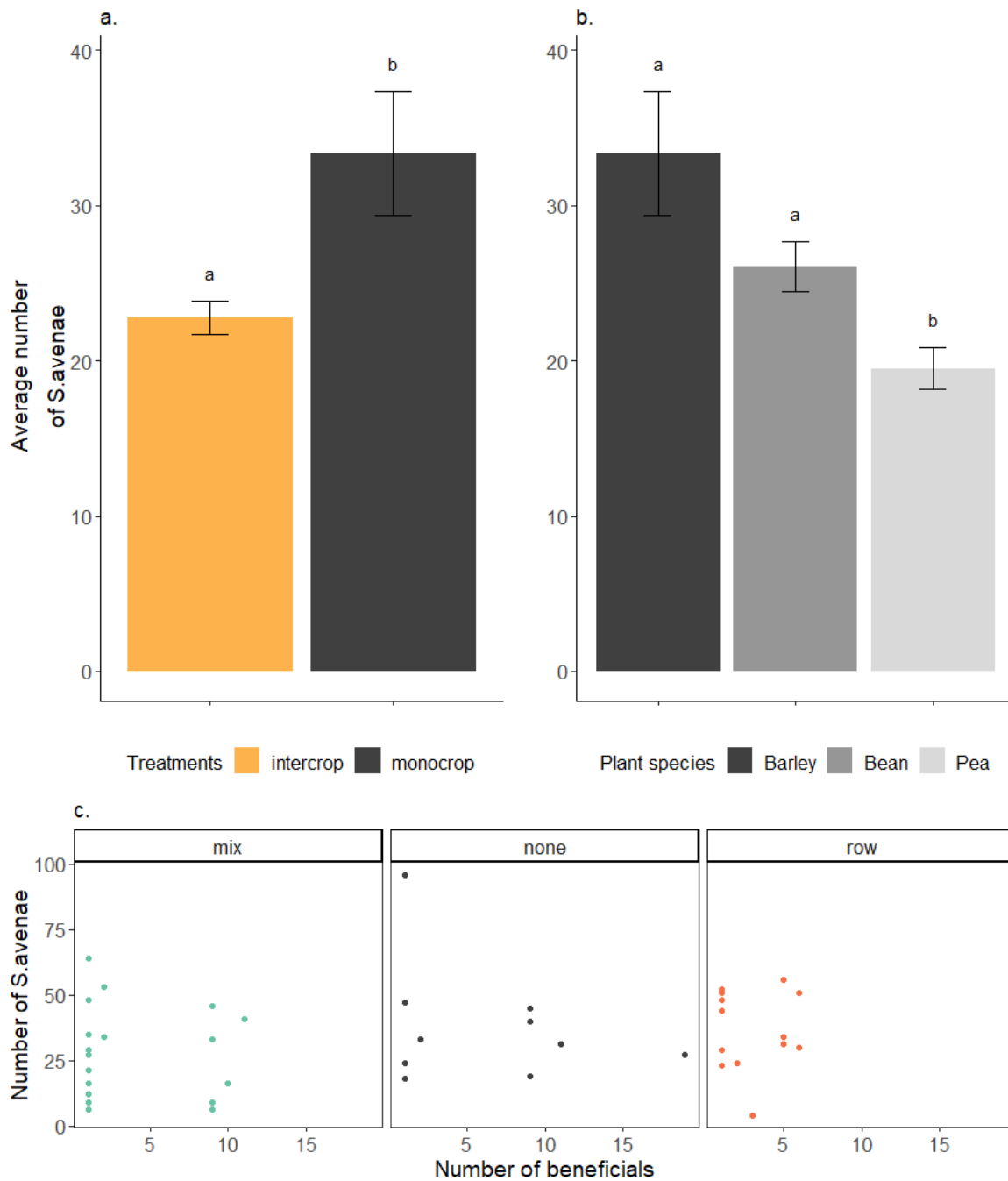


Figure 7: Representation of the number of *S. avenae*, a. between the different treatments, b. between the different plant species intercropped with barley, and c. in relation with beneficial insects number (when present in the plots) and depending on the arrangements (none in the monocrop, row or mix in the intercrop).

Finally the arrangement : plant : number of beneficial insects had no influence on the number of *S. avenae* (Table 1, p\_value = 0.92).

*Peak of the colonies:*

*Intercrop system.*

As found for the results from the development stage described in the section above the number of aphids was significantly different between the treatments for the peak (Table 1 p\_value =

0.0076). The monocrop treatment had a significantly higher number of aphids compared to the intercrops (see Figure 8 a., average =  $161 \pm 11$  and  $118 \pm 5$ , respectively).

The arrangement in the intercrops, plant species and their interaction had no significant influence on the number of *S. avenae* (Table 1, p\_value = 0.33, 0.14 and 0.54 respectively).

#### Presence and number of beneficial insects.

The number of beneficial insects influenced the abundance of *S. avenae* (Table 1, p\_value = 0.0025). Aphids and beneficial abundance are positively correlated, the highest number of beneficial are found where aphids are numerous (Pearson correlation coefficient = 0.26, p\_value = 0.0039).

The interactions between treatment : number of beneficial insects, plant : number of beneficial and arrangement : number of beneficial significantly influenced the model (Table 1, p\_value = 0.017, 0.019 and 0.033 respectively). Figure 8 b. and c. shows that there were fewer aphids and beneficial insects in the intercrops compared to the monocrop. There were more aphids and beneficial insects when only barley was planted, when compared to bean and pea in the intercrops.

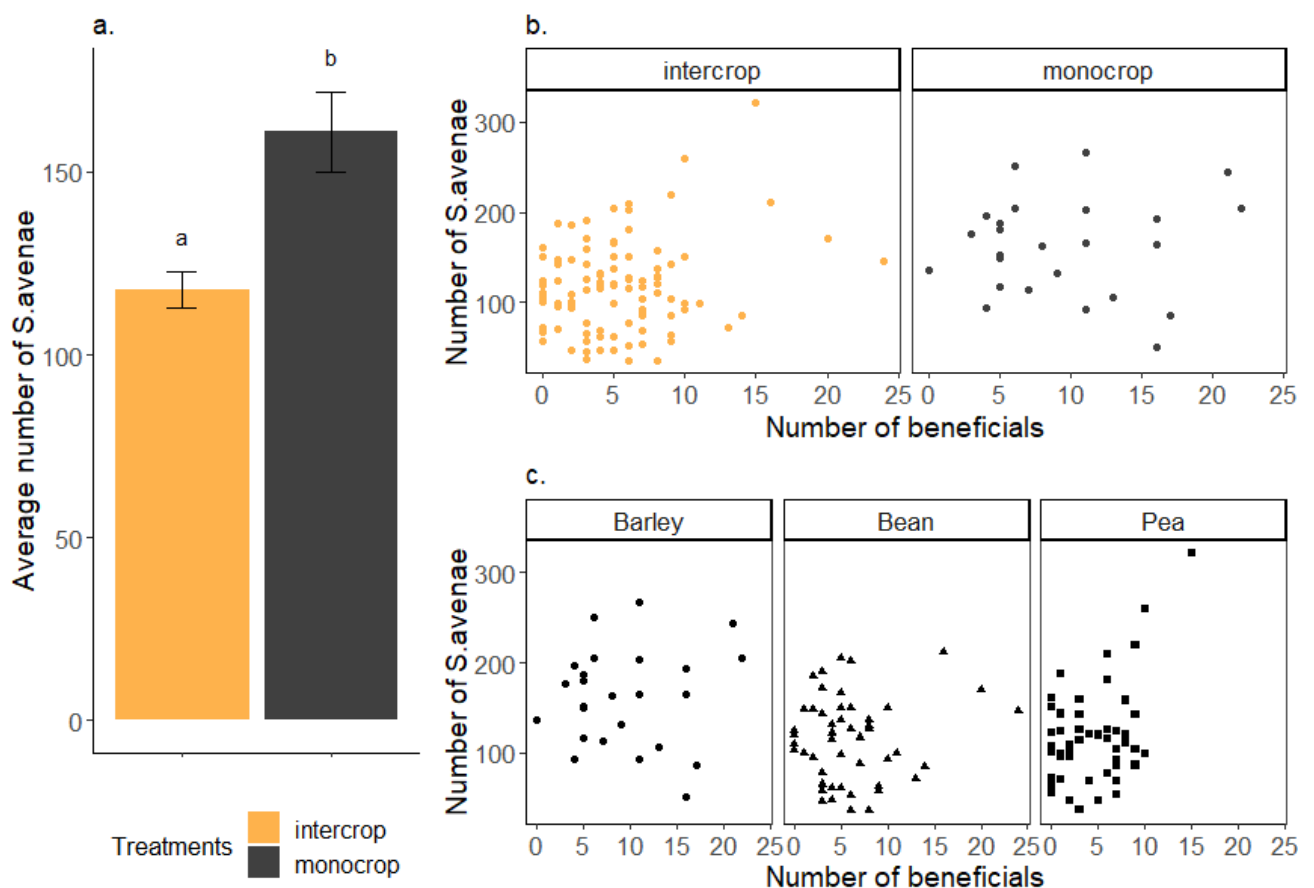


Figure 8: Representation of the statistical analysis. a. average number of *S. avenae* between the different treatments, b. number of aphids in relation with the number of beneficial between the different treatments, c. number of aphids in relation with the number of beneficial between the plant intercropped.

### *Beneficial insect population*

The evolution of the beneficial insect population is highly dependent on the date of counting ( $p\_value = 6.42 \times 10^{-4}$ ). Treatment and arrangement have significantly influenced the number of beneficial ( $p\_value = 0.048$  and  $0.040$  respectively). The monoculture had a higher number of beneficial compared to the intercrops.

Moreover, the interaction plant : arrangement over time was also significant ( $p\_value = 3.65 \times 10^{-4}$ )

## **Discussion**

Evolution of the *S. avenae* population.

No delay was observed in the development of the colonies, contrary to the results found by Helenius (1989). He found a delay in the peak growth for *R. padi* between a bean-oat intercrop and the oat sole crop. In the current study the peak of growth was observed at the same time in each treatment. These differences could be explained by the introduction of *S. avenae* in the field as this design eliminated variables associated with alate colonisation. However, a delay may have been observed in the peak of growth, when flying aphids arrived in the systems. It would be interesting to determine aphid population patterns from natural infestation in the fields, to determine any time laps in aphid colonisation of intercrops.

The number of insects collected in the yellow traps increased over the summer. It is coherent with the increasing number of aphid counted in the field. However, it is unlikely that alate aphids from the yellow trap would have indicated higher influx of cereal aphids to the experiment. Indeed the field was surrounded by woodland, thus the aphid caught would likely be tree aphids.

Parameters influencing the number of aphids:

### *Development of the colonies:*

#### *Intercrop system.*

The intercrop treatments showed a 30% reduction in *S. avenae* number compare to the monocrop treatment. This result is coherent with the result of Zhou et al. (2013) or Lopes et al. (2015). In intercrops of wheat and pea, they found that the intercrops reduced the number of *S. avenae* in the field.

Plant species influenced the number of aphids as pea in the intercrop systems is more efficient than bean in reducing the abundance of aphids when compared to the monocrop. This observation might be explained by the different architecture of the two species. Indeed, beans are straight and unbranched, so they are quite separated from the barley. Peas, however, are crawling and their tendrils wind up around the barley. It might be more difficult for aphids to find their host when it is camouflaged by the intercrop species (Hooks & Fereres 2006) as found in the barley-pea intercrop.

Furthermore, aphids are sensitive to contrasts when they search for their host. When more ground is covered less aphids attack the crop (Irwin *et al.* 2000). In their study, Döring and Röhrig (2016) showed that more aphids landed on traps that were directly on the soil compare to traps put on a green background. In the present study, because the barley-pea intercrops showed better soil coverage compared to barley sole crop and barley-bean intercrops, it can explain that less *S. avenae* were counted. Pea seems to be a good candidate species to be intercropped with barley in reducing *S. avenae* numbers.

Compared to what was expected the number of aphids was not influenced by the arrangements. Thus, row and mix intercropping showed no differences. As, host plants are closer to the others within a row in row intercrops, it was expected that colonies would have grown better in these systems. This result is consistent with Lopes *et al.* (2015), in a wheat-pea intercrop, at the development of the aphid colonies (beginning of their study), the arrangement had no influence on aphid number. Moreover, the plant : arrangement interaction did not influence the number of aphids.

#### *Presence and number of beneficial insects.*

During the development of the colonies few beneficial insects were counted. Yet, more beneficial insects were counted in the pure stand of barley.

#### *Peak of the colonies:*

#### *Intercrop system.*

Intercrop treatments showed a reduction of 27% in aphid number compare to the monocrop. The arrangement did not influence the number of aphids. This result is consistent with Lopes *et al.* (2015) using a wheat-pea intercrop system where they found that for the peak of growth in aphid populations the monocrop had higher number of *S. avenae* on the tiller compared to both row and mix intercrops. Although, they also found that in the last week of their experiment, the mix intercrop was the one with significantly less aphids.

Singh *et al.* (2017) found that the plant combination had no influence on the number of aphids. They intercropped orka with either maize or bean and found similar number of aphids in all the systems. Because arrangement and plant had equal numbers, it was not possible to determine a more efficient intercrop to control *S. avenae*.in the present experiment.

These results support the “*resource concentration*” hypothesis, because more aphids were present in the monocrop system. It can be hypothesised that the intercrop disturbs aphids in finding their host. Soil coverage was similar between treatments, due to crop growth. Although, it is surprising that the pea intercrops were not distinct from the others, as better soil coverage was maintained during the season.

#### *Presence and number of beneficial insects.*

The number of beneficial insects increased during the time aphid colonies were reaching their maximum. Higher number of beneficial insects were counted in the monocrop, where higher number of aphids were also present. These results are coherent with Lopes *et al.* (2015), when

they differentiated beneficial species and found more beneficial insects in the monocrop systems. This result indicates that, although more beneficial insects fed on aphids (observation made in the field) in the monocrops, more aphids were counted there compare to the intercrops.

This is in contradiction with the “*natural enemy*” hypothesis. As found by Zhou et al. (2013) and Xie et al. (2012), it is expected that more beneficial are present in intercrops, because of higher plant diversity. In our study a positive correlation was found between the number of aphids and the number of beneficial insects. When considering beneficial insects, it is coherent with the “*resource concentration*” hypothesis: they are attracted by the area were more food is concentrated. Thus beneficial insects gathered in the monocrop system where they found more aphids.

Although, it can be noted that the different plots were close to each other they were surrounded by a non-weedy grass margin. Thus, flowers from either legumes or weeds in the plots might have attracted the beneficial adults. But these natural enemies would only have laid their eggs in the monocrop. A better understanding in the natural enemies may explain better their action in intercrops. For example, similar experiment at higher scale and with longer distances between the plots may have different results.

#### Summary

In this experiment, it has been demonstrated that:

- i. Intercrops are an efficient strategy for controlling *S. avenae* on barley in the field, as *S. avenae* numbers were reduced in these systems.
- ii. The arrangement of the crop species did not influenced on the number of cereal aphids.
- iii. Pea was interesting to control aphid populations, but for the overall season, the plant intercropped had no influence on the number of aphids.
- iv. Insect natural enemies were more abundant in the monocrop system.

# Chapter 2 : Virus transmission

## Introduction

Viruses are pathogens that can infect living organisms, including plants. Most of the phytopathogenic viruses are found to be transmitted by insects, referred to as vectors (Day & Irzykiewicz 1954). These viruses can be divided in two different categories: the persistent viruses and the non-persistent viruses.

Vector can remain infective for a long time while infected by a persistent, but there is a delay between the time of acquisition of the virus and the time the vector is able to transmit it. When infected by a non-persistent virus, vectors can transmit the virus directly after acquisition but only during a short period of time (Day & Irzykiewicz 1954).

There are few means to reduce virus transmission in the field, the most common being the use of chemical insecticides against the virus vector. With the growing will of reducing chemical use in the field, the use of intercropping to reduce virus transmission is studied. According to the “*virus-sink hypothesis*” intercrops could reduce the losses caused by a virus (Irwin *et al.* 2000).

There are only a few studies on the impact of intercrops on non-persistent virus transmission. Some intercrops don't show any impact of the plant mixture on the virus epidemic. When soybean was intercropped with either sorghum or maize, no differences in Soybean Mosaic Virus (SMV) transmission was observed between the intercrop and the pure stand of soybean (Bottenberg and Irwin, 1992 cited by Irwin *et al.*, 2000). However, a model on the different parameters that can influence virus transmission in the field was developed. Intercrops were taken into account (between soy-bean and other different crops). It is expected that intercrops will reduce the yield losses due to transmission of SMV (Irwin *et al.* 2000). Moreover, it was found that intercropping potato and oat or vetch reduces PVY incidence by 34% and 33% respectively (Dupuis *et al.* 2017).

Potatoes are threatened by PVY, which is a non-persistent virus. It highly damages the crop yield worldwide which implies high economical losses (Valkonen 2007). Symptoms from PVY are yellow mosaic on the leaves, rugosity and malformation of the potato plant (Valkonen 2007). In Scotland potato is an important crop, particularly in the north of the country where seed-potatoes are produced. The PVY tolerance in the potato seed is 0.1% of the total (1 plant in 1,000; SASA, 2013), which indicates the importance of keeping the area free from PVY,



where the seeds are produced. Knowledge of virus epidemiology has been acquired to control the spread within crops.

*Mizus persicae* is the peach-potato aphid. It has a dioecious holocyclic life-cycle (see Figure 2), but is asexual all year in the UK. *M. persicae* migrates to its potato host plant between May and June. It attacks mainly the young leaves when it lands on potato plants (Fraval 1998b).

The aim of the study was to determine the ability of a potato-barley intercrop system to reduce PVY transmission by *M. persicae*, at a greenhouse scale. The hypothesis tested was that *M. persicae* loses its ability to transmit PVY after probing on barley plant.

## Materials and methods:

Experiment 1:

The aim of this experiment was to determine the impact that an intercrop may have on PVY transmission by *M. Persicae*, at an individual scale.

### *Plant material and aphid colonies.*

To understand the abilities of viruliferous aphids to transmit PVY in an intercrop system, infected potato leaves, barley leaves and *Physalis* seedlings were used. The potato leaves were sampled from infected potato plants grown from tuber eyes and planted in a multi-purpose compost (BULLRUSH professional). The barley leaves were sampled from barley plants sown a week before the start of the experiment. The *Physalis* seedlings were sown two or three weeks before. The *Physalis* (from the Solanaceae family as potatoes) was used instead of potato as a receptor plant for the aphids because it is a more susceptible host of the PVY. Virus detection on this species is much quicker and clearer.

Peach-potato aphids *Myzus persicae*, were used here (Kotzampigikis *et al.* 2009). They were kept in colonies, in plastic pots (see the above mentioned design, Figure 5). They were fed with uninfected fresh potato leaves.

### *Treatments.*

We implemented laboratory conditions to mimic intercrops. To do that the aphids were manually transferred from one leaf to the other. A paint brush was used carefully to avoid injuring the aphids. Three treatments were tested, two controls and one intercrop (Figure 9).

For each treatment apterous aphids were starved. Ten aphids were taken from their colonies and put in a 5 ml glass bottle. After 2 hours of starvation they were transferred to an infected potato leaf which was placed in a rectangular plastic box.

After 10 minutes the aphids were transferred:

- Directly to the *Physalis* seedlings with one aphid per seedling (Power 1991). This acted as the positive control.

- For 5 minutes in the plastic box, without any leaves in it (to test if probes on plastic matter can remove the virus from the aphid). Afterwards the aphids were put on the *Physalis* seedlings (one aphid per seedling). It was the negative control.
- For 5 minutes on two barley leaves kept in a plastic box. And then put on the *Physalis* seedlings (one aphid per seedling). This was the intercrop treatment.

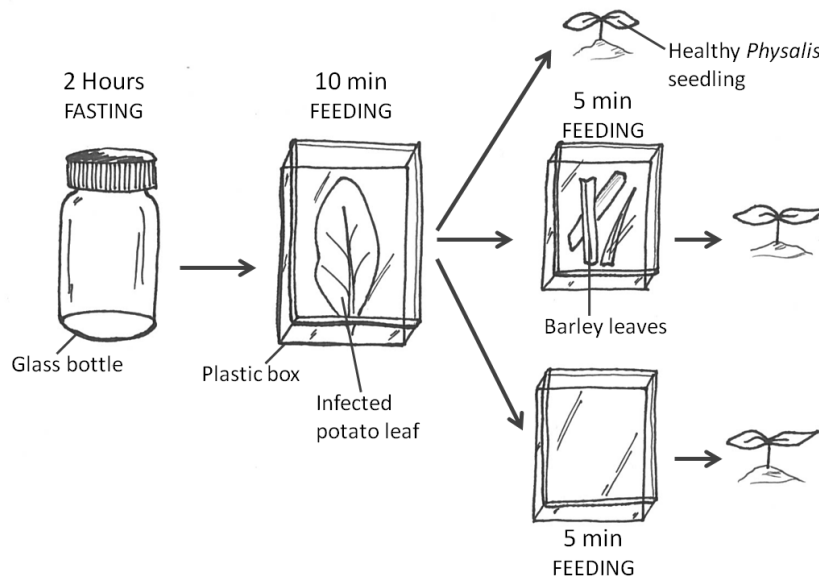


Figure 9: Experimental design of Experiment 1. Times refer to the period for which aphids stayed in the compartment.

Each seedling was covered with a net (see Figure 10) to prevent the aphid escaping.

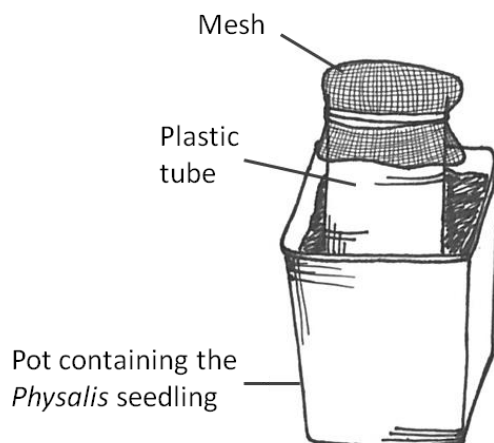


Figure 10: Design of the nets used to keep the aphid in the pot

The aphids stayed on the *Physalis* for one day and then the number of aphids on each seedling was evaluated by visual observation. After that, the *Physalis* were sprayed with insecticides (Hallmark Zeon and Imidacloprid) to kill the aphids. The plants were kept in controlled temperature greenhouses. When the temperature was above 20°C fans automatically switch on

to cool the greenhouse. However, because of a warm summer, the temperature inside the greenhouse was always higher than 20°C.

Three weeks after transmission, the number of *Physalis* infected with PVY was recorded. Four visual observations of the symptoms were made. The whole experiment was replicated 16 times, at different start dates.

#### *Statistical analysis.*

Not all the *Physalis* plants survived until the observations of the symptoms were made. Thus dead plants were removed from the statistical analysis. As the virus symptoms on *Physalis* were observed four times an adjusted average was kept for the statistical analysis, to get binomial data. Some of the results were different from the observations. Thus it was decided that when there was an absence of PVY symptoms in more than half of the observations, the plant was considered free from symptoms.

If less than 5 of the 10 *Physalis* survived until the observation of the symptoms, the whole repetition was removed from the statistical data.

The rate of infected *Physalis* shows a Poisson distribution, thus data were analysed using a generalised linear model (law family: Poisson, link: logit). In addition, the infected potato leaves used in each replicate came from different potato plants. To take into account this difference the leaves were used as a random factor (i.e. the way the infected potato leaf may influence the model will be the same for each 10 aphids that fed on this leaf). The difference between the parameters of the model was tested by an analysis of the variance method.

The model tested the influence of the treatment on the rate of infected *Physalis*:

$$\frac{\text{Number of } Physalis \text{ presenting PVY symptoms}}{\text{Number of survival } Physalis} \sim \text{Treatment} + (1 | \text{Infected leaf used})$$

The model was elaborated using the “lme4” package from the statistical software R version 3.3.2 (2016-10-31), by using the function “glmer”.

An ANOVA (type III) was performed to determine the effect of the treatments factor on the infected *Physalis* rate.

#### Experiment 2:

This experiment was used to determine whether intercropping potato and barley could reduce the spread of the aphids, and reduce the ability of aphids to transmit PVY to healthy plants.

#### *Plant materials and aphid colonies.*

Pots were used to model intercrops. The intercrop system used was potato associated with barley. All the plant material used in these experiments were planted and grown by the SRUC. Infected potato tubers were used; they came from ELISA tested tubers from SASA which had been multiplied in the field and presented PVY symptoms at harvest. The tubers were grown to get potato plants that were later used as virus source for the aphid vectors. The barley was sown in a pot one week before the beginning of the experiment. The whole plants were then transplanted into pots to mimic a possible barrier for aphid dispersal and reduction in transmission. On the last row, *Physalis* was used as a sink and indicator plant. It was sown

three weeks before being transplanted to the pots. All the plants were transplanted into black pots of 23cm diameter and 25cm high. Multi-purpose compost (BULLRUSH professional) was used.

Peach-potato aphids (*Myzus persicae*) colonies were used in this experiment. They were grown on healthy potato leaves in plastic cups (as presented Figure 5), and were kept at a constant temperature of 17.5°C.

#### *Treatments.*

Two different conditions were examined. In the first one, viruliferous aphids were used to determine the impact of the barley on virus transmission. It ensured that the aphids had the virus. In the other, barley's impact on virus transmission was identified by using healthy aphids deposited on an infected potato plant. The direct feeding from aphids on the potato plant was closer to what can happen in natural conditions; aphids moved when ready. For both conditions, we used two different patterns, one to mimic intercrop, and the other one as a control (see Figure 11).

- Condition 1: Twenty previously infected aphids were introduced in the pots, in which no potato plant was present. To get the PVY virus, the aphids were starved for 2 hours, and then fed for 10 min on an infected potato leaf. The 20 aphids infected with the virus were added on a filter paper (approx. 2cm<sup>2</sup>), in the middle of the pots.
  - Control 1: A row of 6 *Physalis* seedlings was planted on the edge of the pot.
  - Treatment 1: A continuous row of about 10 barley plants was planted, and as in Control 1 a row of 6 *Physalis* was on the edge of the pot. The distance between the barley and the *Physalis* was about 5cm.
- Condition 2: Non-viruliferous aphids were added to the pots, in which a potato plant was grown. In these pots 20 aphids, starved for 10 min, were added on the potato plant. They were put on the infected potato so that they could receive the virus and then transmit it to other plants.
  - Control 2: A potato plant was in the centre of the pot, surrounded by a row of 6 *Physalis*, planted on the edge of the pot.
  - Treatment 2: The same design as Control 2 in which a row of barley was added. About 10 barley seedlings were transplanted between the potato and the *Physalis*; approximately 5 cm distance from both plants.

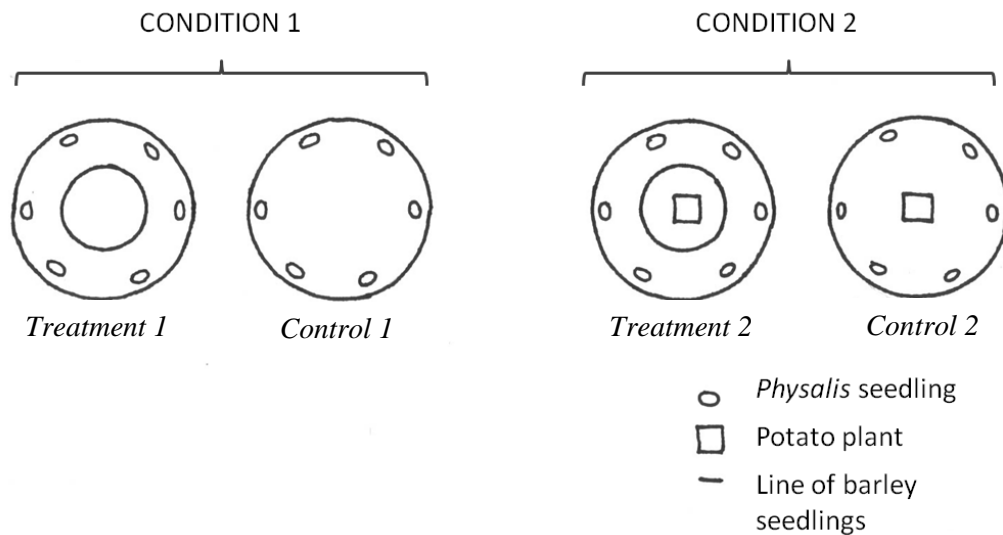


Figure 11: Experimental design of the pots for Conditions 1 and 2

On top of each pot nets were used to prevent the aphids escaping (see Figure 12). All the pots were placed in a greenhouse and organised in a latin-square design. The greenhouse was temperature-controlled by fans, which switched on when the temperature exceeded 20°C.

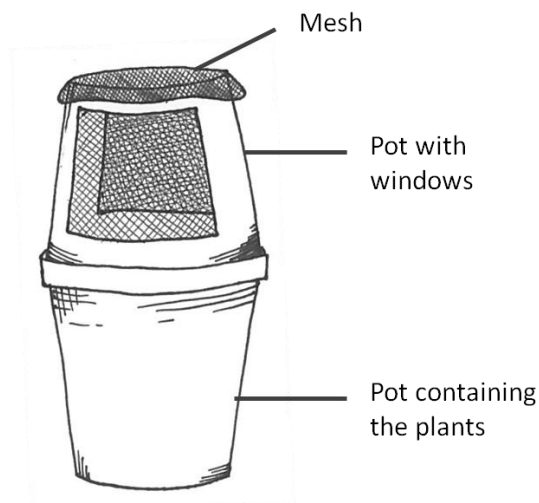


Figure 12: Pot design during the experiment.

#### Counting and observations.

The pots were kept for two weeks and three counts were made and adults, winged aphids and wingless nymphs were differentiated. After two weeks the aphids were chemically killed with Hallmark Zeon and Imidacloprid, and each *Physalis* was transplanted in an individual pot which was grown on and scored for virus symptoms, three times after the transplant. This was to determine whether the aphids had been able to transmit PVY. Symptoms (see Figure 13) were identified when the plant was weak or small presenting curled leaves and yellow mosaic on them.

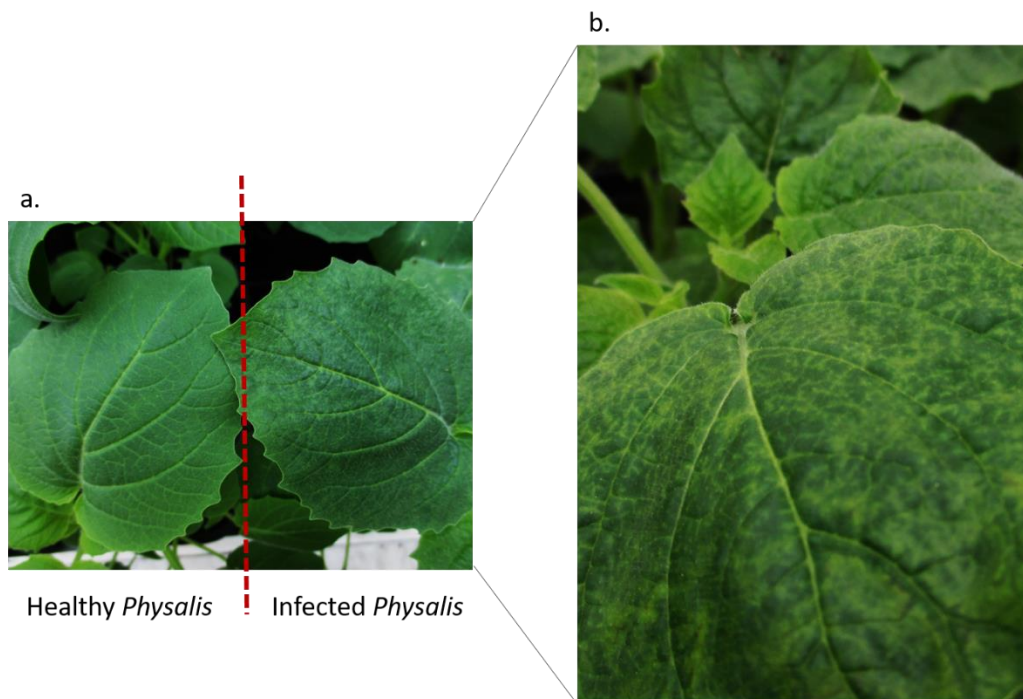


Figure 13: Picture showing in a. the differences between a healthy *Physalis* (on the left) and a PVY infected one (on the right). In b. is a zoom of an infected *Physalis*. Both pictures are contrast and light modified to increase the visibility of the symptoms.

#### Statistical analysis.

Data were analysed in terms of population behaviour and virus transmission for clearer understanding. Moreover, as aphids from the two conditions got the virus in a different manner, they were analysed separately.

#### Population dynamics:

The data of aphid number present in the pots did not follow a Gaussian distribution, but showed a Poisson distribution. This suggested the use of a generalised linear model was appropriate (law family: Poisson, link: logit).

The experiment was repeated 3 times, and because the greenhouse conditions may have been different between them, heterogeneity brought by the repetitions was taken into account in the. Then, the counting was a repeated measurement in time, each pot was different from the others. As this difference was similar between the measurements, and illustrated the growing of aphid's colonies, it was decided to put the counting times as a random factor. Furthermore, heterogeneity between the pots in each replicate was included in the model. To do that, the interaction date of start : pots was used as a random factor.

The difference between the parameters of the models was tested by analysis of variance method. In all models the influence of the treatment was taken into account.

On the one hand, the influence of intercrop on the total number of aphids was tested for both conditions (1 and 2):

$$\text{Number of aphids} \sim \text{Treatment} + (1 \mid \text{Counting.time}) + (1 \mid \text{Date.start} : \text{Pot})$$

Then, to determine whether the barley could act as a physical barrier to the aphid dispersion only the number of aphids present on the *Physalis* was used in the analysis. Yet, number of aphids present on the *Physalis* and total number of them in the pot were the same for Control 1, thus no statistical analysis was made in this case. However, in Condition 2 the total number of aphids in the pot might have influenced the number of aphids that were on the *physalis*, so it was put in the model in addition to the treatments.

$$\begin{array}{l} \text{Number of} \\ \text{aphids} \\ \text{on the Physalis} \end{array} \sim \text{Treatment} * \begin{array}{l} \text{Total} \\ \text{number} \\ \text{of aphids} \end{array} + (1 | \text{Counting.time}) + (1 | \text{Date.start} : \text{Pot})$$

#### Virus transmission:

As in the experiment 1 dead *Physalis* were removed from the data analysis and adjusted average of the virus symptoms observations were used. So we had binary data. Binomial distribution defined the model, and Date.start : Pot was a random factor.

The analysis of variance tested the difference between the treatments, in each condition. The model used was as followed:

$$\begin{array}{l} \text{Number of physalis} \\ \text{presenting PVY symptoms} \end{array} / \begin{array}{l} \text{Number of survival} \\ \text{physalis} \end{array} \sim \text{Treatment} + (1 | \text{Date.start} : \text{Pot})$$

The model was elaborated using the “lme4” package from the statistical software R version 3.3.2 (2016-10-31), by using the function “glmer”.

An ANOVA (type III) was performed to determine the effect of the parameters in the models.

## Results

### Experiment 1

All the treatments had an even number of infected *Physalis* compare to the number of plant tested, p\_value = 0.8. Figure 14 shows a slightly different result compare to the two controls, although the difference is not significant.

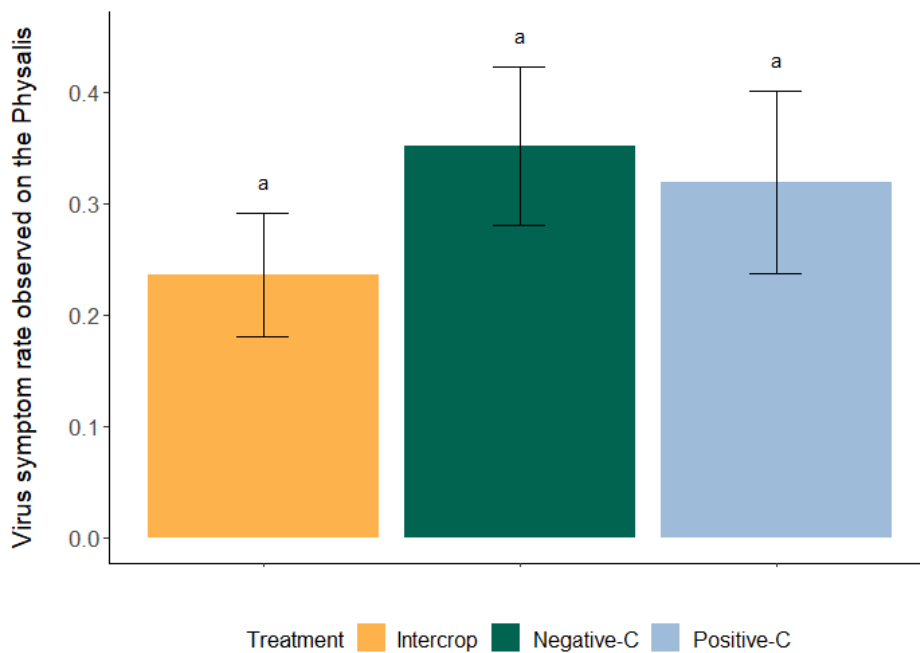


Figure 14: Rate of infected physalis (i.e. number of *Physalis* presenting PVY symptoms / total number of *Physalis* per replicate) in each treatment.

## Experiment 2

### Population distribution

For all treatments, the number of aphids increased between the three counting date (Figure 15, a. and c.). The results are then divided between Condition 1 and Condition 2.

#### Condition 1:

The number of aphids was significantly ( $p\_value \ll 0.05$ ) higher in the intercrop treatment compared to the control without barley (average number of aphids per pot =  $54 \pm 8$  and  $70 \pm 9$ , respectively).

#### Condition 2:

The same trend as the Condition 1 was observed for the total number of aphids. It was significantly higher ( $p\_value \ll 0.05$ ) in the intercrop treatment compare to the control without barley (average number of aphids per pot =  $121 \pm 16$  and  $159 \pm 19$ , respectively). The number of aphids that reached the *Physalis* plants was significantly different between the treatments ( $p\_value = 0.0033$ , Figure 15 b.). It was also highly influenced by the total number of aphids present in the pot ( $p\_value = 2.4 \cdot 10^{-8}$ ). However the interaction treatment : number of aphids in the pot was not significant.



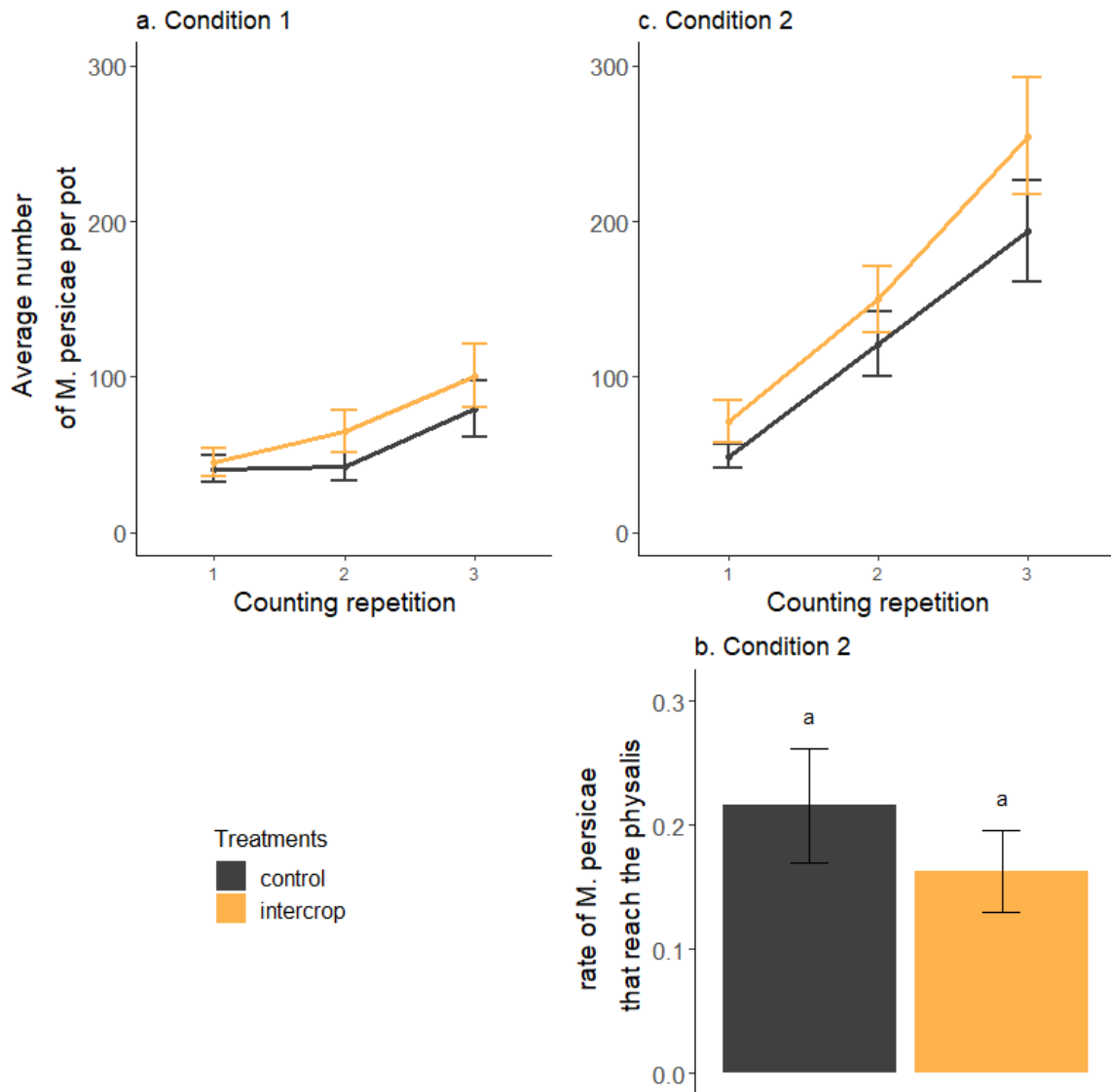


Figure 15: Average number of *M. persicae* present in the pots. a. and c. represent the increase of the population between the three counts (without (Condition 1) or with (Condition 2) a potato plant in the pot, respectively). b. represents the number of aphids that reached the *Physalis*. The letters indicate the influence of the treatment in the model, when letters are identical the treatments had no influence on the number of aphid that reached the *Physalis*.

#### Virus transmission

In both conditions the prevalence of infected *Physalis* was not different between the two treatments (Figure 16,  $p_{\text{value}} = 0.15$  without potato in the pot,  $p_{\text{value}} = 0.21$  with). Transmission rate was  $0.25 \pm 0.06$  in the Condition 1 and  $0.27 \pm 0.05$  in the Condition 2.

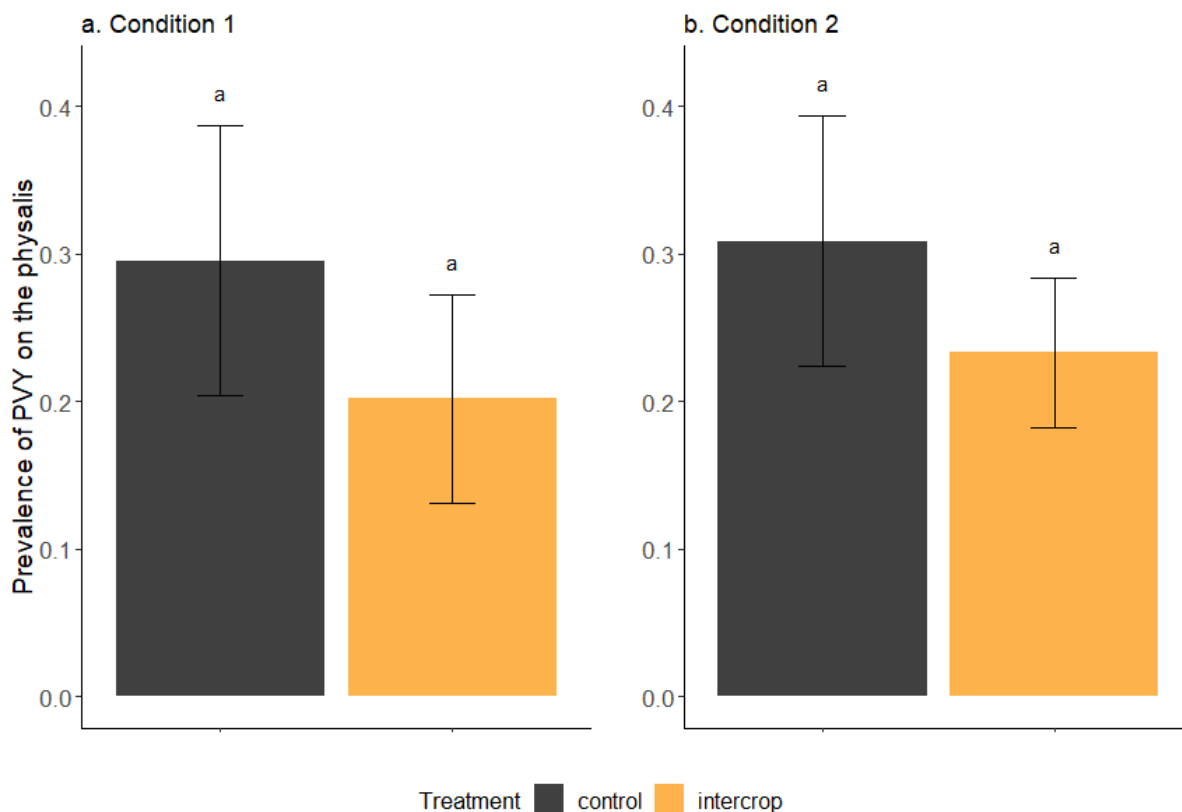


Figure 16: Prevalence of PVY in the *Physalis* plants. a. refers to the results when no potato was present in the pot and b. refers to the results when a potato plant was in the pot. Letters on the graphs refer to the differences between the modalities. When letters are the same, there is no statistical differences.

## Discussion

### Experiment 1

The two controls (i.e. with or without time laps between transfer of aphids from the potato leaf to the *Physalis*) used in these experiments were expected to show high rates of virus symptoms. But Figure 14 shows that less than half of the *Physalis* plants were infected in these treatments. Thus, the transmission rate was not high in this experiment. However, no DAS-ELISA test were performed (as it was done by Power et al. (1991) or Kotzampigikis et al. (2009)), so latent infected *Physalis* (i.e. carries the virus without showing symptoms) were not detected.

Both controls showed an even symptom rate on the *Physalis* which indicated that the plastic box had no effect on virus transmission. It seems that the aphids were either not probing the plastic or during these probes aphids did not lose the virus. Then, because the aphids from both the intercrop and the negative control treatments stayed the same time on each steps before being put on the *Physalis*, it is unlikely that time (between acquisition and transmission) had an influence on the virus transmission in these experiments.

To sum it up, the intercrop has no effect on reducing virus transmission in this experiment.

## Experiment 2

### *Population distribution*

The increase in number of aphids in the pot between the three counts can be explained by the building of the colonies.

All the intercrop treatment presented higher number of aphid compared to their respective control. This is in contradiction with the results bound by Lopes et al. (2015) and Lai et al. (2017), but coherent with the one of Helenius (1989) and Dupuis et al. (2017). However, the colonies seemed to be influenced by the size of the plants, as explained before aphids are sensitive to the density of individuals on a plant (Dixon 1985).

Indeed, influence of the total number of individuals was shown, for both conditions, when the number of aphids present on the *Physalis* was tested. Thus analysing the interaction between treatment and number of aphid on the *Physalis* has more meaning compare to the treatment itself. It could be an indicator of any barrier effect from the barley on the aphid dispersal.

In the Condition 1 the interaction [*treatment : total number in the pot*] was not taken into account as the number of aphid on the *Physalis* was the same as the one in the whole pot in Control 1. In this case, the fact that the treatments influence the number of aphid on the *Physalis* can only be explained by the presence of barley. The intercrop has no significant influence, although it had a lesser number of aphids on the *Physalis* compare to its control ( $52 \pm 7$  and  $54 \pm 8$  respectively). Thus we can deduce that the intercrop has no barrier effect on the aphid dispersion.

In Condition 2 the results were similar. The interaction [*treatment : total number in the pot*] had no influence on the number of aphid on the *Physalis*. This indicates that no barrier effect was detected on aphid spread.

### *Virus transmission*

As found in experiment 1, intercrops were not reducing the PVY transmission rate compared to their control. This is not coherent with results from Dupuis et al. (2017), for whom the intercrop reduced PVY transmission by aphids.

This result may not be related to the feeding time of aphids on potato leaves. Although, it is accepted that to acquire and be able to transmit the virus only brief probes are effective (Pirone & Perry 2002). Moreover (Kotzampigikis *et al.* 2009) found that the longer an aphid feeds on an infected plant the lesser is its ability to transmit the virus later. In the present study, similar prevalence of PVY was found between the two conditions (1 and 2). Non-previously infected aphids may have fed for longer periods on potato leaves while in the pot, but it might not have any negative effect on their virus transmission ability.

When taking into account the aphid population, it can be argued that as the intercrop had no barrier effect on aphid dispersal: the same number of aphids reached the *Physalis* (Figure 15 b.). The even quantity of aphids could be, in this case, the explanation of the even transmission rate. For instance Mondal et al. (2016) found that the prevalence of PVY was positively related with the number of aphid captures in the field.

When considering the “*virus sink*” hypothesis (Irwin *et al.* 2000), it seems that aphids did not probe on the barley before reaching the *Physalis*.

Why intercrops were not efficient in controlling virus transmission?

Both experiments resulted in the same conclusion on intercrops between potato and *Physalis*. The introduction of barley within a potato crop is not an efficient system to control PVY transmission by *M. persicae*. Indeed no differences were observed between treatments in either conditions.

It is possible that barley is not attractive to *M. persicae*, which consequently went directly to the *Physalis*. Either put directly on barley (experiment 1), or passed it (experiment 2) to reach the *Physalis*, it is possible that aphids did not probe the barley. Indeed, sensory information is necessary for aphids to select a host (Powell *et al.* 2006; Schröder *et al.* 2017). For example, potato leaves were pubescent (i.e. leaves have a hairy surface) whereas barley was glabrous (i.e. without hair on its leaf surface). As the leaves of barley did not match the plant characteristics aphids were looking for, they probably decided not to probe on these.

Furthermore, potato and barley might emit different volatile compounds. Yet aphids are able to distinguish volatiles, some being attractant while other repellent (Powell *et al.* 2006; Bruce & Pickett 2011; Schröder *et al.* 2017). In their study, Nottingham *et al.* (1991) found that *Aphis fabae* used plant volatiles to locate and select their host. They also found that aphids are able to distinguish mixtures of these plant volatiles. In the present results, it is possible that either the potato emitted attractive compounds or barley emitted repellent ones. Moreover, (Rajabaskar *et al.* 2014) found that viruliferous aphids prefer non infected plants. The *Physalis* might have been more attractive in the experiments, as the aphids were viruliferous.

It seems that intercrop might not be used as a control to PVY transmission. However, only apterous aphids were used in this chapter. Thus it might be of interest to investigate the role of the alates, especially because they are the ones that can transmit the viruses over long distances (Schröder *et al.* 2017). Indeed, intercrops might not have influence on wingless aphids, but might disturb landing of winged ones.

Summary

Intercropping potatoes with barley is not efficient to reduce PVY transmission by *M. persicae*.

# Conclusion

Theories about intercrops suggest that these system may be used as aphid control. It is likely that with a greater plant diversity within one field, either aphids will have more difficulties in finding their hosts or aphid's natural enemies will be more abundant. Although intercrops provide agronomic advantages, their ability to control insect pest infestations remains unclear.

The present study showed that the introduction of a legume crop in association with barley reduced by 30% the number of cereal aphids. Between the different intercrop systems, the arrangement (in rows or in random mixture) and the legume species (either pea or bean) were not different. Thus intercrop is a good method to control aphids, however it was not possible to determine whether it was due to a barrier effect or not.

Mummies were present in the field, they indicated that parasitism from parasitoids wasps existed. Syrphid's and ladybird's larvae were also found in the field, and their observation showed that they were directly feeding on aphids. Thus parasitoids and predators were active during the studied period. Aphid's natural enemies were more abundant within the barley sole crop compared to all the intercrops.

Furthermore, intercropping barley with potato did not reduce the prevalence of PVY. In these experiments intercrops were not efficient to reduce virus spread.

To conclude, barley-bean/pea intercrops can be used as a control against *S. avenae*, whereas potato-barley is not a good system to reduce PVY transmission. It has to be carefully implemented, and be used in association with other practices in order to insure an efficient pest control.

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# Appendix



*Evolution in time of the aphid population in the field. The darker is the color the higher the number of *S. avenae* was found. The time scale is based on the barley development stages. Monocrop refers to the barley monocrop. RB-bean and RB-pea refer to Row intercrop of barley, with either bean or pea (respectively). MB-bean and MB-pea refer to Mix intercrop of barley with either bean or pea.*



## CAN INTERCROP CONTROL APHID POPULATIONS AND THEIR ABILITY TO SPREAD NON-PERSISTENTLY TRANSMITTED VIRUS

Insects, in particular aphids, can cause important damage to crops. Until now, chemicals are the most efficient, and widely used method to reduced infestation. Yet, new regulations require good environmental practices, to protect soil and human health. To comply with these, the introduction of higher crop diversity within one field might be a solution. Intercrops (i.e. association in time and space of different crops in a field) have mostly been studied for its agronomic advantages (increase in yield, enhancement of soil properties, economic benefits). However, only a few studies have focussed on the impact of intercrops towards pests. In the present study, implemented in the SRUC-Aberdeen campus, field and greenhouses experiment were carried out on the ability of intercrop to either reduce aphid infestation or non-persistent virus transmission. Barley-legume intercrops were compared to a barley sole crop. The system tested both plant species (bean or pea) and arrangement (row or mix) toward *Sitobion avenae*. All intercrops showed a 30% reduction in aphid numbers during the whole experiment. Intercrops with pea were more efficient to reduce *S. avenae* numbers at the development of the colonies. However, no distinction between the intercrops was observed at the peak of the colonies. Although a higher number of beneficial was found in the barley sole crop, intercrops seem to be efficient to control aphid development in the field. PVY transmission from *Myzus persicae* in a potato-barley intercrop showed no differences compare to sole potatoes. In this system intercrop was not efficient to reduce virus spreading.

Key-words: Intercrops, cereal-legume, *S. avenae*, virus transmission, PVY, *M. persicae*