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Sarah Grauby

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Sarah Grauby. Diversification végétale intra-parcellaire et régulation biologique des pucerons des céréales, vecteurs du virus de la jaunisse nanisante de l'orge. Agronomie. AgroParisTech, 2023. Français. NNT: 2023AGPT0003 . tel-04121799

HAL Id: tel-04121799

<https://pastel.hal.science/tel-04121799v1>

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THESE DE DOCTORAT

pour obtenir le grade de

Docteur d'AgroParisTech

Spécialité : Agro-écologie

École doctorale n° 581

Agriculture, alimentation, biologie, environnement et santé (ABIES)

par

Sarah GRAUBY

Diversification végétale intra-parcellaire et régulation biologique des pucerons des céréales, vecteurs du virus de la jaunisse nanisante de l'orge

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Thèse présentée et soutenue à Lyon, le 03 mai 2023

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Avant-propos

Ce projet de thèse a débuté en septembre 2019 et a été effectué au sein de l'unité de recherche Agroécologie et Environnement de l'Institut Supérieur d'Agriculture Rhône-Alpes (ISARA) à Lyon. Il a été financé par le projet européen H2020 Ecostack.

Différents travaux de valorisation sont issus de ce travail de thèse.

Communication orale dans un colloque national

Grauby, S., Tolon, V., Roume, A., Wezel, A., Jacquot, E. and Ferrer, A. (2022). Inter-specific crop diversification and bottom-up regulation of *Rhopalosiphum padi*, vector of the Barley yellow dwarf virus. In: *12^{ème} réunion du réseau "Biologie Adaptative des Pucerons et Organismes Associés"*, 17-18th of May 2022, Nice, France.

Grauby, S., Tolon, V., Roume, A., Wezel, A., Ferrer, A. and Jacquot, E. (2023). Cereal intercropped with a clover cover and bottom-up regulation of *Rhopalosiphum padi*, vector of the Barley yellow dwarf virus. In: *RVV2023 - 19^{ème} rencontres de virologie végétale*, 15-19th of January 2023, Aussois, France.

Communication sous forme de poster dans un colloque national

Grauby, S., Roume, A., Wezel, A. and Ferrer, A. (2021). Barley variety mixture and association with a clover cover: effects on cereal aphid populations and their generalist natural enemies. In: *Entomo2021 - 42^{èmes} journées des Entomophagistes*, 8-10th of November 2021, Rennes, France.

Grauby, S., Wezel, A., Ninkovic, V., Ferrer, A. and Jacquot, E. (2023). Impact of plant diversification in barley fields on aphid populations and yellow dwarf disease prevalence. In: *RVV2023 - 19^{ème} rencontres de virologie végétale*, 15-19th of January 2023, Aussois, France.

Publications scientifiques dans une revue internationale à comité de lecture

Grauby, S., Ferrer, A., Tolon, V., Roume, A., Wezel and A., Jacquot, E. (2022). Can Mixed Intercropping Protect Cereals from Aphid-Borne Viruses? An Experimental Approach. *Insects*, *13*, 521.

Stages co-encadrés

Kratz Garreth, 2020. Master thesis. Double degree Wageningen University and Research - Agroecology and Environment ISARA. "Effect of intra- and inter-specific diversification on aphid pest and its natural enemies in organic barley cropping systems".

Arnoult Aurore, 2021. Master 2, Université de Tours. "Effets de la diversité végétale intra et inter-spécifique sur l'abondance des carabes et sur la régulation biologique des pucerons en culture biologique d'orge d'hiver".

Esteves de Oliveira Guilherme Patrick, 2022. Master 2, L'Institut agro Montpellier. "Inter-plant movement of cereal aphid species which occupy different positions on their host plant".

Remerciements

Si j'ai réussi à mener ce travail jusqu'au bout, c'est que de nombreuses personnes m'y ont aidée, à de nombreux moments et de nombreuses manières. Je souhaiterais les remercier ici :

À mes encadrants Alexander Wezel et Aurélie Ferrer, pour m'avoir choisie et fait confiance pour mener à bien ce projet. Merci à Aurélie pour ses relectures et ses conseils.

À Emmanuel Jacquot, pour son aide et son soutien. Sa rigueur, sa détermination, sa grande pédagogie m'ont beaucoup apporté. À son équipe, qui m'a accueillie pendant trois mois à Montpellier. Un merci particulier à Marlène Souquet et à Thomas Armand.

À Anthony Roume, pour son aide précieuse, ses réflexions toujours avisées. Pour sa vision de la science, son approche naturaliste.

À Vincent Tolon, qui m'a fait découvrir son monde des statistiques avec entrain et patience. Aux discussions passionnantes sur la science, l'agroécologie, l'humain.

À mon comité de thèse, Joséphine Peigné, Velemir Ninkovic, Gaël Thebaud et Manuel Plantegenest. Merci à eux pour leur aide lors des comités et leur disponibilité pour de nombreux échanges.

Aux membres de mon jury de thèse pour avoir accepté d'évaluer ce travail.

À tous mes collègues de l'ISARA, qui ont rendu mon séjour là-bas agréable.

À mes stagiaires, qui ont été des expériences humaines incroyables. À leur motivation sans faille pour compter des pucerons, à braver des conditions climatiques difficiles, à m'aider dans les pires galères automobiles dans les chemins agricoles. Merci à Léna, Violaine, Garreth, Pierre, Aurore et Guilherme.

Aux agriculteurs qui ont accepté de mettre en place nos essais sur leur terre. À toutes nos discussions et leurs visites inopinées. Au travail qu'ils font au quotidien, dans des conditions complexes. Mr Vacher, Mr Jury, Mr Barral, Mr Gourdain, Mr Walter, Mr Colombier, Mr Barioz, Mr Perret, Merci.



À mes amis, que je ne pourrai tous citer ici. À ceux présents particulièrement pendant cette période : Alice, Chloé, Fred, Violaine, David, Moebis, Michou, Julo, Carolina, Coco et les Grandjacques. Aux Rostang, amis de toujours.

À Corinne. Les mots me manquent tant l'aide qu'elle m'a apportée est immense.

À la famille Salque et leur chère Haute-Loire. Jean-Pierre, Jacqueline, Solène, Romain, Caro, Pierre et bébé Julia, je vous dois beaucoup.

À toute ma famille. À ma Manou, dont l'amour et le soutien qu'elle me porte diffusent en moi, même à distance. À mon papy, dont les souvenirs partagés me portent. À grand-père et grand-mère. Aux Bessons, qui m'ont soutenue durant toutes ces années et qui m'ont tous à leur manière inspirée.

À Clémentine qui a été d'un grand soutien. Qui de petite sœur se retrouvait en grande conseillère.

À ma mère, cette grande artiste, qui a préféré ne pas faire de son talent son métier pour garder pure la flamme qui l'habite. À la petite échelle de ce manuscrit, je souhaite mettre ses œuvres dans la lumière.

À mon père, ce grand scientifique, à qui j'ai tenté de marcher dans les traces. Trente ans après, je finis à mon tour ce long et laborieux travail.

À Jean-Marie, pour m'avoir fait découvrir d'autres voies. Pour son soutien et sa patience. À toutes les belles choses à venir.

À la nature, que j'observe, qui m'apprend, qui m'inspire,

À cette victoire sur la peur,

À mes parents, à ma sœur,

À ma bête,

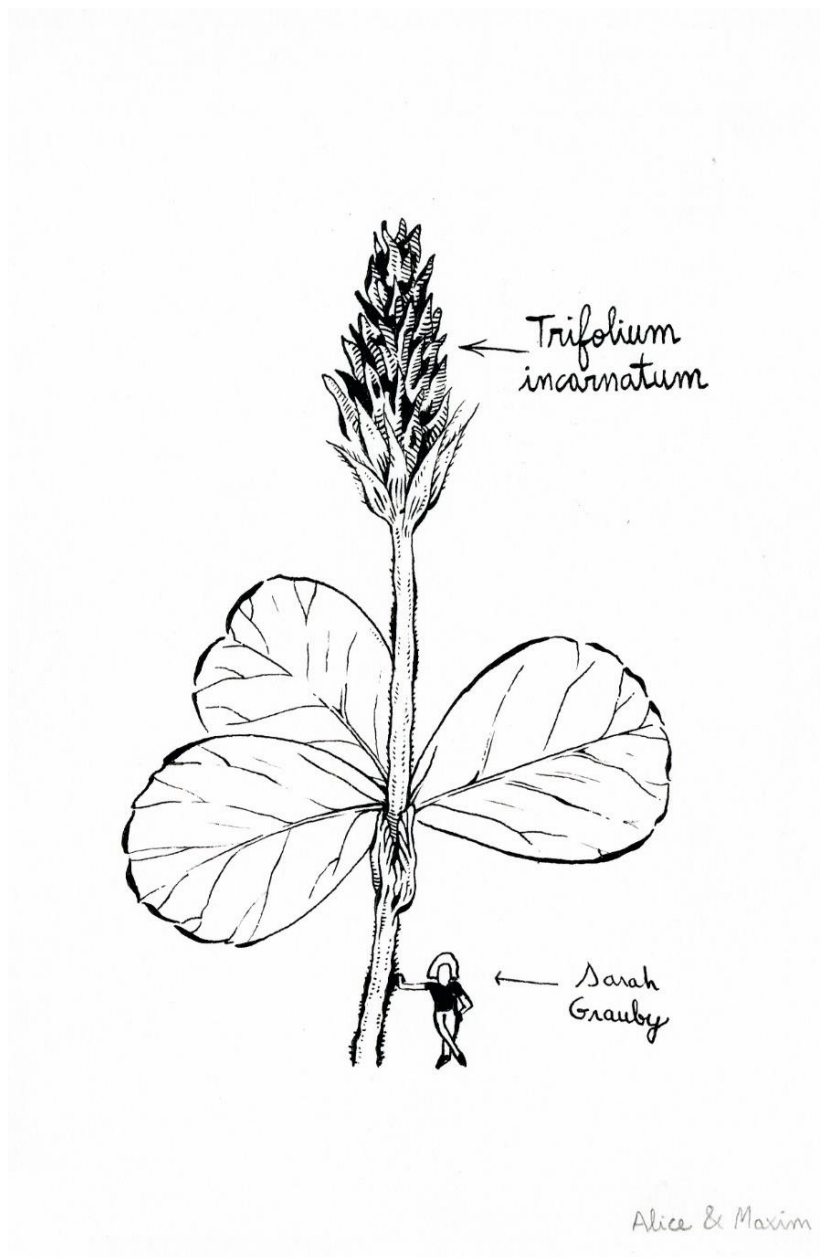


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CHAPITRE 1

INTRODUCTION GENERALE

Aquarelle, syrphe ceinturé et colonie de pucerons
Epsyrphus balteatus
Christine Grauby

Chapitre 1. Introduction générale

Partie 1. Contexte général

1. Limites de l'agriculture productiviste

L'intensification de l'agriculture, initiée lors de la révolution verte dans les années 1960 a permis une augmentation très importante des rendements agricoles, permettant de nourrir une population mondiale croissante (Matson *et al.*, 1997; Tilman *et al.*, 2002). Cependant, cette agriculture productiviste, par la mécanisation et l'utilisation de nombreux intrants (fertilisants, pesticides, irrigation) a causé des impacts environnementaux néfastes dans les agroécosystèmes sur les compartiments eau, sol, et air (Carpenter *et al.*, 1998; Tilman *et al.*, 2002; Naylor, 1996; Vitousek *et al.*, 1997). Cette intensification de l'agriculture a également contribué à une diminution de la biodiversité (Matson *et al.*, 1997; Tilman *et al.*, 2002), avec la perte simultanée de fonctions écologiques associées telles que la pollinisation et le contrôle biologique (Poveda *et al.*, 2008). Les agroécosystèmes intensifs sont également devenus très sensibles aux infestations d'insectes ravageurs des cultures (Landis *et al.*, 2000). Cette grande sensibilité s'explique principalement par une perte de biodiversité végétale à plusieurs échelles :

i) à l'échelle de l'agroécosystème : par l'augmentation de la taille des parcelles et de leur agrégation, par l'uniformisation des stades végétatifs, par des assolements et des rotations simplifiées, et par la réduction des habitats favorables aux ennemis naturels des ravageurs (Jervis *et al.*, 2004; Deguine et Ratnadass, 2013).

ii) à l'échelle de la parcelle : par la culture en pure d'une seule espèce/variété (dont certaines variétés améliorées sont plus sensibles aux ravageurs), qui représentent une ressource de nourriture pure et concentrée pour le ravageur. Les techniques de désherbage, de fertilisation et d'irrigation des cultures, amplifient également la sensibilité des cultures pures aux ravageurs (Deguine et Ratnadass, 2013).

Ainsi, face à ces impacts négatifs importants de l'agriculture productiviste, le défi majeur actuel de l'agriculture est d'évoluer vers un mode de production plus durable, conciliant la nécessité de produire en quantité suffisante et celle de préserver les agroécosystèmes et leurs fonctions (Matson *et al.*, 1997).

2. La diversification végétale : un pilier de l'agroécologie

L'agroécologie, discipline scientifique et approche interdisciplinaire (Wezel *et al.*, 2009), propose deux axes majeurs pour répondre aux enjeux de production et de durabilité des agroécosystèmes (Deguine et Ratnadass, 2013) :

- i) l'incorporation de la biodiversité végétale dans les agroécosystèmes.
- ii) l'amélioration du fonctionnement biologique des sols.

L'agroécologie permet en s'appuyant sur ces deux axes majeurs, de concevoir des agroécosystèmes économes en intrants, moins sensibles aux maladies et aux insectes ravageurs (Malézieux *et al.*, 2009) où productivité et durabilité sont compatibles (Deguine et Ratnadass, 2013).

2.1. Les pratiques de diversification végétale dans les agroécosystèmes

La diversification végétale peut être introduite dans les agroécosystèmes à travers différentes pratiques et à différentes échelles spatiales et temporelles (Tibi *et al.*, 2022) (Figure 1.1).

A l'échelle de la parcelle, la diversification intra-spécifique (mélange variétal) et la diversification inter-spécifique (association d'espèces) peuvent être mises en place. Les bandes enherbées, bandes fleuries et haies permettent d'introduire de la diversité végétale en bordure de parcelles agricoles. Puis, la diversification végétale peut également être mise en place à l'échelle des rotations, en les allongeant et en intégrant un nombre plus important d'espèces cultivées (Meynard *et al.*, 2013). A l'échelle du paysage, le niveau de diversité végétale, dans sa composition (nature des cultures présentes dans l'assolement) et dans sa configuration (taille et forme des parcelles, répartition spatiale des cultures) résulte des choix réalisés à l'échelle de l'exploitation (Figure 1.1).

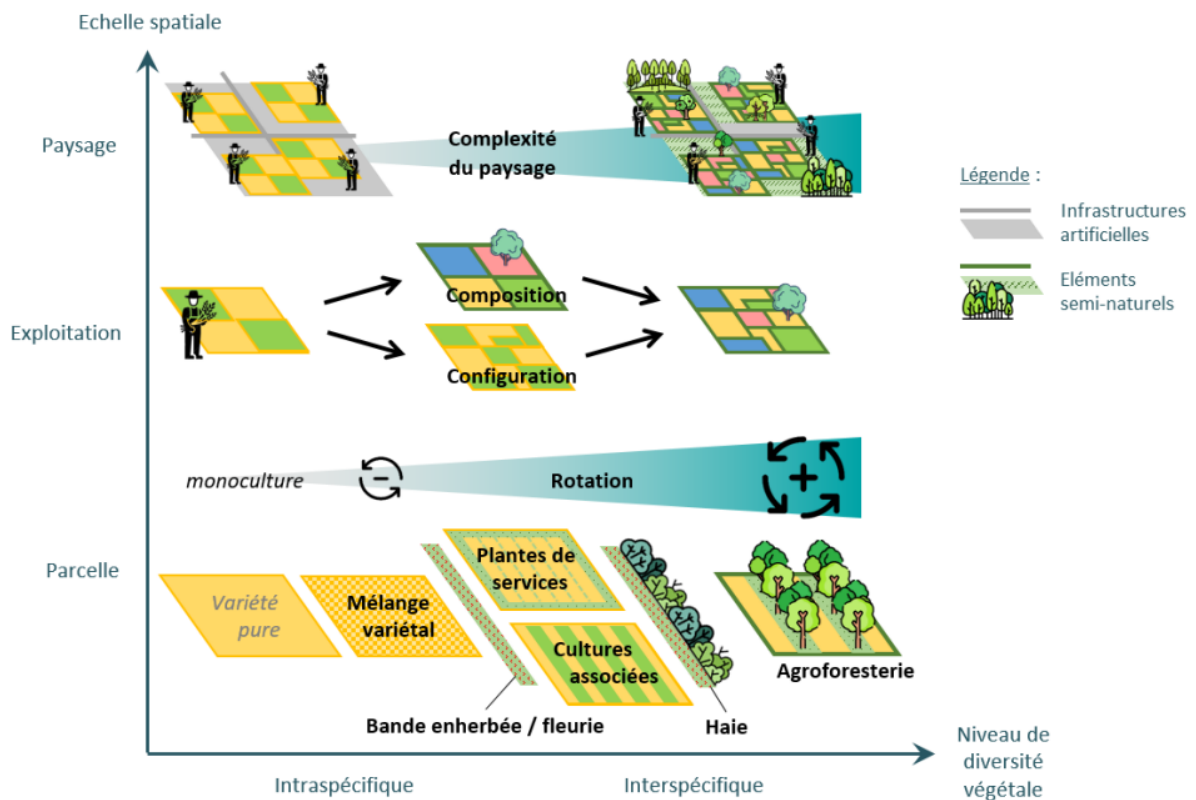


Figure 1.1. Pratiques de diversification végétale et mise en œuvre à différentes échelles spatiales et temporelles. (Source : Tibi *et al.*, 2022).

2.2. La diversification végétale et régulation des insectes ravageurs

De manière générale, les insectes herbivores spécialistes sont moins abondants en cultures diversifiées par rapport à des cultures non diversifiées (Andow, 1983; Risch *et al.*, 1983; Andow, 1991; Hooks et Johnson, 2003). Des méta-analyses ont observé une diminution des populations d'insectes en cultures diversifiées dans 70% (Muriel et Velez, 2004) et 53% (Podeva, 2008) des articles examinés (350 et 62 articles publiés respectivement). Letourneau *et al.* (2011) ont quantifié dans une méta-analyse ce contrôle biologique des insectes nuisibles en cultures diversifiées : ils ont estimé que la diversification permettait en moyenne une augmentation de 44% de la présence d'ennemis naturels et de 54% de la mortalité des insectes ravageurs. Récemment, Beillouin *et al.* (2021) ont conduit une méta-analyse de 95 méta-analyses publiées (« second-order meta-analysis ») et ont également conclu à une augmentation du contrôle biologique des organismes nuisibles (maladies et ravageurs) en cultures diversifiées de 63% en moyenne. D'autres services de régulation sont aussi augmentés par la diversification végétale comme la qualité de l'eau (+51%), la qualité des sols (+11%), mais aussi le service de production avec 14% d'augmentation des rendements (Beillouin *et al.*, 2021).

Hypothèses de mécanismes sous-jacents

Plusieurs hypothèses ont été avancées pour expliquer la diminution des insectes spécialistes sur leur plante hôte en cultures diversifiées par rapport aux cultures non diversifiées.

Plusieurs d'entre elles reposent sur des effets directs de la diversité végétale sur l'insecte par des mécanismes bottom-up :

(i) Une plante non-hôte peut rendre la plante hôte plus difficilement localisable par l'insecte (« disruptive-crop hypothesis ») (Vandermeer, 1989), par différents types de perturbations. Lors d'une perturbation olfactive, la plante non-hôte peut masquer (Tahvanainen et Root, 1972) ou altérer l'odeur de la plante hôte (Theunissen, 1994). Lors d'une perturbation visuelle, la plante non-hôte peut masquer visuellement la plante hôte (Smith, 1969; 1976). Puis, lors d'une perturbation physique, la plante non-hôte par sa végétation, peut représenter une barrière (en largeur ou hauteur) qui entrave les déplacements de l'insecte (Perrin et Phillips, 1978).

(ii) Une plante non-hôte peut également attirer l'insecte en étant plus attractive que sa plante hôte (« trap-crop hypothesis ») et ainsi l'éloigner de cette dernière (Vandermeer, 1989).

(iii) Enfin, une plante non-hôte peut repousser l'insecte, en émettant des composés volatiles répulsifs (Uvah et Coaker, 1984; Vandermeer, 1989; Khan *et al.*, 2006).

Une autre hypothèse avancée est celle d'un effet indirect de la diversité végétale sur l'insecte via des mécanismes top-down, où la plante non-hôte recrute les ennemis naturels de ce dernier (« natural enemy hypothesis ») (Root, 1973). En effet la plante non-hôte peut fournir des ressources alimentaires (e.g. proies/hôtes alternatifs, pollen, nectar) ou un habitat (e.g. refuge, site d'hivernation), favorables aux ennemis naturels du ravageur.

Cependant, dans beaucoup d'études, ces mécanismes restent des hypothèses pour expliquer une diminution du nombre d'insectes nuisibles dans une situation de culture diversifiée par rapport à une monoculture et les études ne testent pas toutes expérimentalement le mécanisme *stricto sensu*. De plus, pour une même situation de régulation biologique, plusieurs mécanismes pourraient intervenir en même temps (Finch et Collier, 2000).

3. La diversification végétale intra-parcellaire

3.1. La diversification intra-spécifique

La diversification intra-spécifique consiste à augmenter la variabilité génétique du peuplement cultivé d'une espèce, en semant plusieurs variétés simultanément (mélange variétal) ou en utilisant des variétés plus hétérogènes (variétés traditionnelles ou paysannes) (Tibi *et al.*, 2022). Les mélanges variétaux sont aujourd'hui principalement utilisés par les agriculteurs pour réguler les maladies fongiques (complémentarité des variétés vis-à-vis des pathogènes) mais aussi pour stabiliser les rendements. L'utilisation de mélanges variétaux de blé est récente en France, mais a rapidement augmenté, passant de 1% des surfaces en 2007 à 12% en 2019 (Montazeaud *et al.*, 2022).


Effets sur les bioagresseurs

La littérature scientifique étudiant l'effet de la diversification intra-spécifique sur la régulation des pathogènes aériens est abondante selon l'ESCo (expertise scientifique collective conduite par l'INRAE) et le consensus d'un effet positif est très fort (Tibi *et al.*, 2022, Table 1.1). Concernant les insectes aériens, la littérature est relativement importante, avec un consensus pour un effet positif de ce type de diversification sur ces derniers.


Table 1.1 Synthèse des effets des deux types de diversification végétale intra-parcellaire sur les différentes catégories de bioagresseurs issue de l'expertise scientifique collective conduite par l'INRAE (ESCO). (Source : Tibi *et al.*, 2022). **NB** : L'ESCO a réalisé l'analyse d'un important corpus bibliographique (1900 références dont 80% d'articles scientifiques) pour évaluer si la diversité végétale des espaces agricoles peut favoriser la régulation naturelle des bioagresseurs des cultures.

	Adventices	Insectes aériens	Insectes telluriques	Maladies vectorielles	Pathogènes aériens	Pathogènes telluriques	Nématodes	Autres bioagresseurs
Mélanges variétaux	*	**	?	*	***	*	?	?
Cultures associées	***	***	*	?	***	*	?	?


Nature de l'effet de la modalité de diversification sur la régulation du bioagresseur concerné:


 **Consensus de la littérature en faveur d'un effet positif** sur la régulation du bioagresseur.
(Plus le vert est foncé, plus le consensus est fort)


 **Hypothèse théorique** sur l'existence d'un effet

 **Information insuffisante** pour conclure (y compris sur le plan théorique)

Niveau d'investigation de ces effets dans la littérature:

 Littérature **rare/très peu abondante** (mais suffisamment robuste pour conclure)

 Littérature **relativement abondante** mais pas/très peu de travaux de synthèse/reviews

 Littérature **abondante** incluant des travaux de synthèse/reviews

3.2. La diversification inter-spécifique

3.2.1. Définition et services

La diversification inter-spécifique, ou « intercropping », consiste à cultiver simultanément plusieurs espèces végétales au sein d'une même parcelle. Si les espèces sont toutes des cultures de rente (finalité de production de biomasse ou de grain), on parle alors de cultures associées ou « true intercropping ». Dans le cas où la plante associée n'est pas une culture de rente mais vise plutôt à fournir d'autres services à la culture principale (contrôle des adventices et des ravageurs, amélioration de la fertilité des sols, *etc.*), on parle alors de plante compagne ou de plante de service (Tibi *et al.*, 2022).

Effets sur les bioagresseurs

La littérature scientifique étudiant l'effet de la diversification inter-spécifique sur la régulation des adventices, des insectes phytophages et des pathogènes aériens est abondante selon l'ESCO et il y a un consensus fort en faveur d'un effet positif de ce type de diversification sur ces trois bioagresseurs des cultures (Tibi *et al.*, 2022, Table 1.1). Parmi cette littérature scientifique, l'association céréale-légumineuse est particulièrement étudiée. On remarque toutefois, que l'effet de la diversification inter-spécifique sur les maladies vectorielles (i.e. transmises par vecteur) reste méconnu (Table 1.1).

Couvert « multi-services » : exemple de l'association céréale-légumineuse

L'utilisation des associations céréale-légumineuse est en forte augmentation en France, notamment en agriculture biologique. En six ans, les surfaces cultivées ont été multipliées par 10 (5 000 ha en 2010 à 50 000 ha en 2016) (Moutier, 2021). Selon le débouché de la légumineuse, les services attendus de l'association varient. En élevage, les associations (e.g. triticale-pois fourrager) permettent de produire un ensilage productif, stable face aux aléas climatiques, riche en matière azotée et en fibre et économe en intrants (azote, produits phytosanitaires et eau) (Protin *et al.*, 2009).

Pour le débouché grain (e.g. blé-pois protéagineux), les associations permettent de bons rendements avec de bonnes teneurs en protéines, en limitant très fortement les intrants azotés par rapport à la culture d'une céréale en pure (Naudin *et al.*, 2010; Bedoussac et Justes, 2010; Pelzer *et al.*, 2012). Concernant l'utilisation d'une légumineuse en plante compagne, celle-ci apporte également de nombreux services : régulation des adventices et autres bioagresseurs, contribution au stock d'azote organique du sol, amélioration de la structure du sol (Corre-Hellou *et al.*, 2013). Après la récolte de la céréale, la légumineuse peut continuer à couvrir le sol jusqu'au semis de la culture suivante.

3.2.2. Différents types d'arrangements spatiaux et temporels

Selon les services attendus par l'association de cultures, différents arrangements spatiaux des cultures associées peuvent être mis en place (Andrews et Kassam, 1976) (Figure 1.2a) :

- i) le "mixed intercropping" où la culture de rente et la culture de service sont cultivées sans arrangement spatial distinct.
- ii) le "row intercropping" où la culture de rente et la culture de service sont cultivées en rangs alternés.
- iii) le "strip intercropping" où plusieurs rangs de la culture de rente sont alternés avec plusieurs rangs de la culture de service.

Différents arrangements temporels des cultures associées peuvent être mis en place (Gardarin *et al.*, 2022) (Figure 1.2b) :

- i) le « synchronous/simultaneous intercropping » où les deux cultures sont semées en même temps et ont des cycles de développement similaires. Si la plante de service n'a pas terminé son cycle après la récolte de la culture de rente, elle peut être détruite ou être maintenue jusqu'à la prochaine culture.
 - ii) le « living mulch » où la culture de rente est semée dans une culture de service déjà établie.
 - iii) le « relay intercropping » où la culture de service est semée dans une culture de rente déjà établie.
- Après la récolte de la culture de rente, la culture de service continue à se développer.

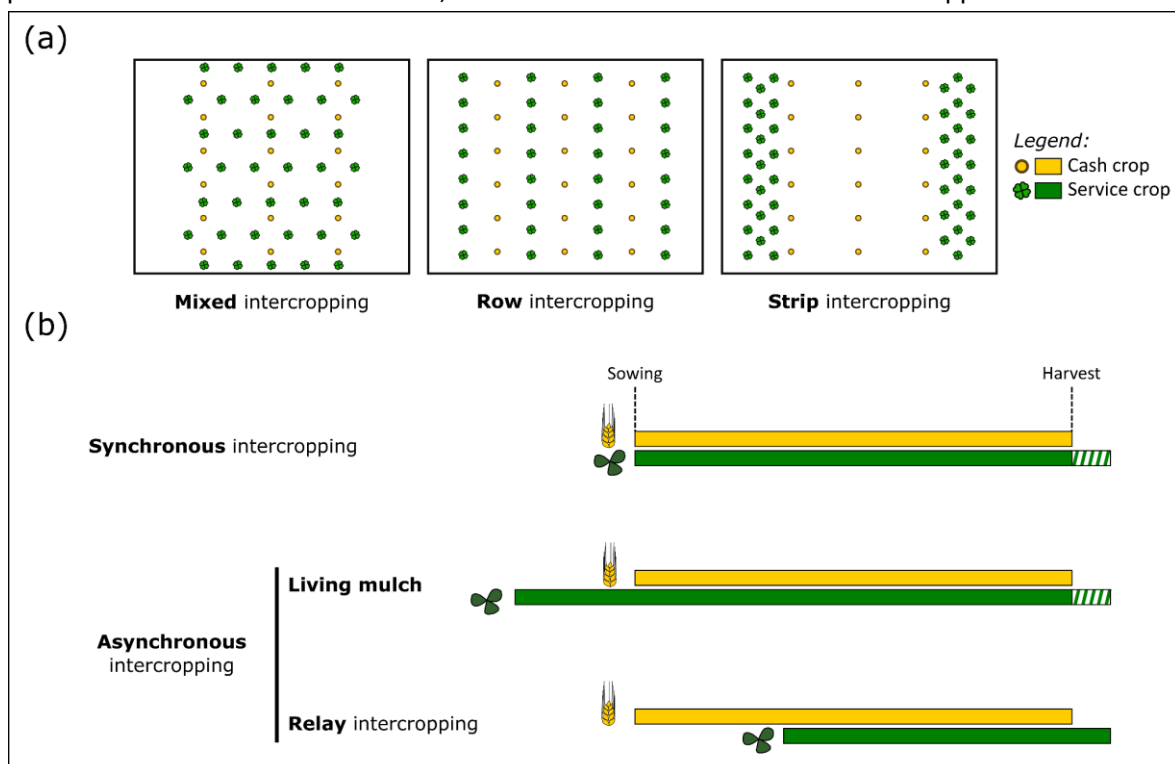


Figure 1.2. (a) Différents arrangements spatiaux et (b) temporels en cultures associées. (Source graphique : Sarah Grauby).

Partie 2. Modèle biologique : les pucerons des céréales

1. Principales espèces de pucerons des céréales en climat tempéré

Parmi les nombreuses espèces de pucerons (Hemiptera : Aphididae) présentes sur céréales, les six espèces suivantes sont considérées comme étant les espèces majeures (Blackman et Eastop, 2000) : *Sitobion avenae* (Fabricius) (grain aphid), *Rhopalosiphum padi* (Linnaeus) (« bird cherry-oat aphid »), *Metopolophium dirhodum* (Walker) (« rose-grain aphid »), *Rhopalosiphum maidis* (Fitch) (« corn leaf aphid »), *Schizaphis graminum* (Rondani) (« greenbug aphid »), *Diuraphis noxia* (Kurdjumov) (« Russian wheat aphid »). Parmi ces derniers, *R. padi*, *S. avenae* et *M. dirhodum* sont les principaux vecteurs de la jaunisse nanisante de l'orge en Europe (Plumb, 1995) (voir la partie 3.2 de cette partie).

2. Cycle biologique des pucerons des céréales

2.1. Holocyclie et anholocyclie

Les pucerons des céréales accomplissent des cycles biologiques complexes comprenant une phase de reproduction sexuée (femelle sexuée ovipare) et/ou une phase de reproduction asexuée (femelle asexuée vivipare ; par parthénogénèse). Un cycle biologique complet, ou holocyclie, implique la présence d'une génération sexuée et de plusieurs générations asexuées par an (Figure 1.3). Selon les espèces de pucerons, cette alternance de phases de reproduction s'effectue sur des plantes hôtes appartenant i) à la même famille botanique (espèces de pucerons monœciques), c'est le cas de *S. avenae* qui réalise tout son cycle annuel sur les poacées ; ou ii) à des familles botaniques différentes (espèces de pucerons diœciques), c'est le cas des espèces *R. padi* et *M. dirhodum*, qui alternent entre hôte primaire (*Prunus padus* en Europe pour *R. padi* et *Rosa spp.* pour *M. dirhodum*) et hôte secondaire (poacées) durant leur cycle biologique (Figure 1.3).

Durant le printemps et l'été, de nombreuses générations de pucerons virginipares aptères sont produites par parthénogénèse sur poacées permettant un accroissement rapide des populations (Figure 1.3). En automne, des conditions environnementales initient le développement de nouvelles femelles parthénogénétiques, les sexupares. Ces dernières donnent naissance à des pucerons mâles ailés et des gynopares ailées, qui accomplissent la migration vers l'hôte primaire. Par parthénogénèse, les gynopares donnent naissance à des femelles ovipares qui sont fécondés par les mâles ailés (Figure 1.3). À l'issue de la fécondation, un œuf est pondu sur l'hôte primaire sur lequel il passe l'hiver en diapause, constituant une forme de survie pendant les conditions climatiques hivernales défavorables. Au printemps, l'œuf éclot et la femelle aptère qui en est issue, la fondatrice, est à l'origine d'une ou plusieurs générations de pucerons parthénogénétiques (fondatrigènes) qui se développent sur l'hôte primaire (Figure 1.3). Puis, des fondatrigènes ailées quittent la plante d'hiver et colonisent les hôtes secondaires, les poacées. De nouveau, un cycle annuel commence. Pour *S. avenae*, l'ensemble du cycle annuel est réalisé sur les poacées. Les œufs sont pondus sur les chaumes de céréales ou à la base des tiges de poacées. Le reste du cycle est très similaire à celui des espèces diœciques précédemment explicité (Figure 1.3) (Hullé *et al.*, 2020).

Certaines espèces de pucerons, qu'elles soient monœciques ou diœciques, peuvent se reproduire toute l'année sous forme de reproduction asexuée (anholocyclie). Cette anholocyclie est favorisée par la douceur de l'hiver ou la rareté des hôtes primaires dans le paysage. Pour une même espèce, des populations holocycliques et anholocycliques peuvent coexister. Les individus passant l'hiver sous forme de larve ou d'adulte sont plus sensibles aux basses températures que les œufs. Ainsi, lors d'hiver doux, les populations anholocycliques pourront survivre et continuer à se multiplier.

Au contraire, lors d'hivers rigoureux, elles seront fortement diminuées, alors que les populations holocycliques survivront grâce aux œufs résistants au froid (Hullé *et al.*, 2020).

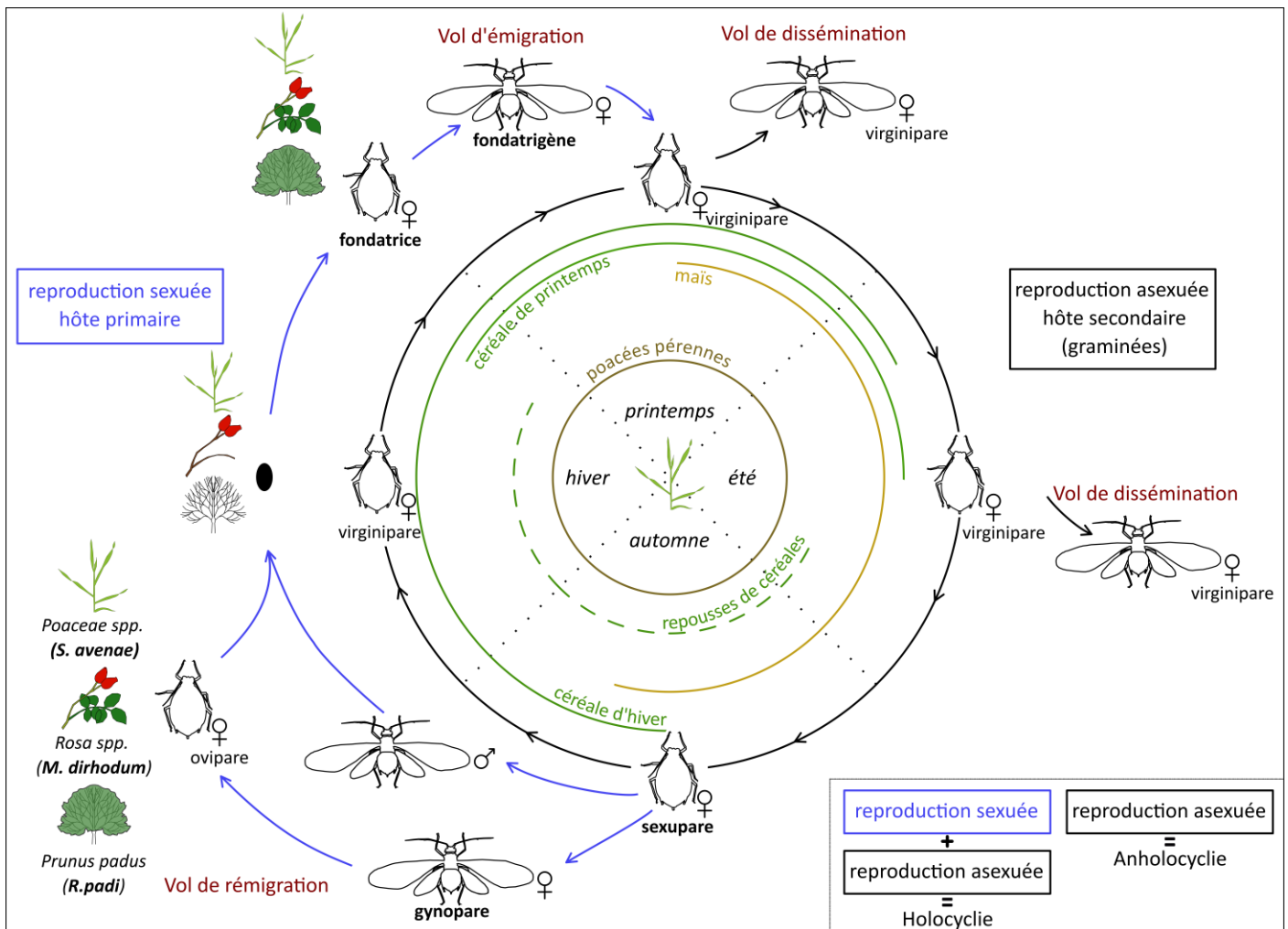


Figure 1.3. Cycle biologique simplifié des pucerons des céréales diœciques (*R. padi*, *M. dirhodum*) et monœcique (*S. avenae*) et cycles des poacées dans le paysage agricole. Cycle holocyclique diœcique (reproduction asexuée sur hôte secondaire + sexuée sur hôte primaire) et cycle anholocyclique (que reproduction asexuée). (Source graphique : Sarah Grauby).

2.2. Activité de vol et alternance des plantes hôtes

Les pucerons des céréales, réalisent ainsi la totalité (espèce monœcique holocyclique ou espèce anholocyclique) ou une grande partie (espèce diœcique holocyclique) de leur cycle biologique sur les poacées. Au cours d'une année, la composition en poacées cultivées varie dans le paysage agricole, à mesure que ces dernières sont semées et réalisent leur cycle de développement. En climat tempéré, les céréales à paille, principalement le blé et l'orge, peuvent être semées en automne (septembre à novembre) et au printemps (mi-février à mi-mars) et sont récoltées en été (juin à juillet). Le maïs est semé de mi-avril à mi-mai et récolté de fin août à octobre (Figure 1.3). Ainsi au cours de la saison, les pucerons des céréales alternent entre différentes plantes hôtes, grâce aux vols de dissémination effectués par les formes ailées. Lors d'une discontinuité entre cultures (e.g. entre la récolte du maïs et le semis des céréales d'hiver), les poacées sauvages ou les repousses de céréales peuvent également être des hôtes pour les pucerons des céréales.

Les trois principales espèces de pucerons présentent différentes périodes d'activité au cours de la saison (Figure 1.4). *S. avenae* présente une activité de vol principalement printanière et estivale. Son activité de vol est maximale fin juin/début juillet (vols de dissémination), puis les vols régressent jusqu'à la fin juillet ou début août, à la suite de la maturation des épis et des récoltes des céréales à paille. *M. dirhodum* a également une activité de vol principalement printanière et estivale. Les captures de juin à début août correspondent à la colonisation des parcelles de maïs depuis les parcelles de blé ou d'orge, qui arrivent en sénescence (Turpeau *et al.*, 2011). Pour *R. padi*, les vols de juin à juillet correspondent à des vols de dissémination des colonies sur céréales à paille, poacées sauvages et maïs (plante hôte majeure de *R. padi*). Puis l'activité automnale de l'espèce de septembre à mi-novembre correspond à la fois au vol de rémigration vers son hôte primaire (populations à reproduction sexuée) et au vol de dissémination vers les poacées sauvages, repousses et jeunes semis de céréales d'hiver (Turpeau *et al.*, 2011).

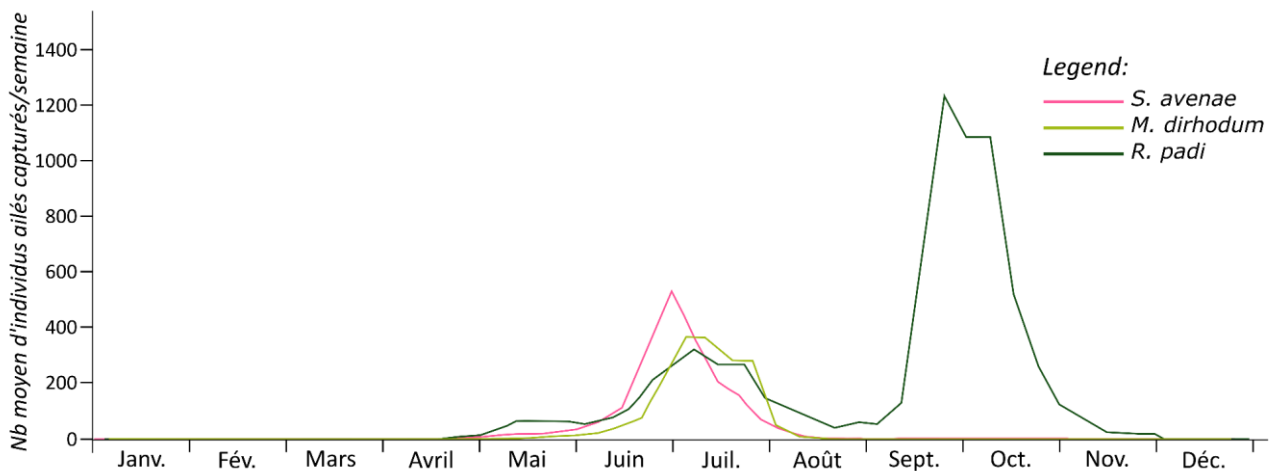


Figure 1.4. Activité de vol des trois principales espèces de puceron des céréales en France. (Source : Turpeau *et al.*, 2011, données hebdomadaires moyennes de 1978 à 2005, réseau Agraphid).

3. Dégâts des pucerons sur céréales cultivées

3.1. Dégâts directs des pucerons : prélèvement de sève

Les pucerons sont des insectes phytophages à pièces buccales de type piqueur-suceur. Ces dernières sont formées d'un rostre entourant deux paires de stylets souples, qui eux même délimitent les canaux salivaires et alimentaires. Ces pièces sont adaptées pour pénétrer les tissus végétaux jusqu'à atteindre les faisceaux criblo-vasculaire du phloème (Figure 1.5) et à prélever la sève élaborée dont les pucerons se nourrissent. Lorsque le puceron insère ses stylets dans la plante, avant l'ingestion du phloème, il injecte de la salive qui forme un manchon autour de ses stylets, limitant leur contact avec les tissus de la plante et permettant au puceron de les manœuvrer. Ensuite d'autres salivations ont lieu, dont une salivation dans les vaisseaux du phloème (Tjallingii, 2006). En prélevant de la sève élaborée, le puceron détourne une partie des éléments nutritifs nécessaires à la croissance et à la reproduction de la plante. Ainsi, il peut entraîner une diminution de la croissance de la plante et une mauvaise fructification. De plus, la sécrétion salivaire est souvent toxique pour le végétal et peut provoquer des destructions et déformations des tissus végétaux.

Les trois espèces de pucerons des céréales ont des sites d'alimentation différents sur leur plante hôte. *S. avenae* se nourrit préférentiellement sur les épis des céréales et peut ainsi provoquer des pertes de rendement. En effet, le nombre de grains par épi peut être réduit si les populations sont importantes au moment de la floraison. Si les populations sont importantes au stade remplissage du grain, c'est la qualité du grain qui est altérée (Johnston et Bishop, 1987; Kolbe et Linke, 1974).

Les dégâts directs des espèces se nourrissant sur le feuillage, tel que *R. padi* ou *M. dirhodum* sont moindres que celles se nourrissant sur les épis (Niehoff et Stäblein, 1998).

3.2. Dégâts indirects des pucerons : vecteurs de virus

3.2.1. Le pathosystème de la jaunisse nanisante de l'orge

Les pucerons des céréales sont responsables de la jaunisse nanisante de l'orge (JNO), une des maladies virales sur céréales les plus importantes et répandues dans le monde (D'Arcy, 1995).

Espèces virales

Cette maladie est causée par dix espèces virales formant le complexe « Barley/Cereal yellow dwarf viruses » (B/CYDV). Les espèces virales causant la JNO appartiennent aux familles virales des *Solemoviridae* (genre *Polerovirus*, espèces : CYDV-RPV, CYDV-RPS et MYDV-RMV ; Scheets *et al.*, 2020), des *Tombusviridae* (genre *Luteovirus*, espèces : BYDV-PAV, BYDV-PAS, BYDV-MAV, BYDV-kerII et BYDV-kerIII ; Scheets *et al.*, 2020) ou encore non assignées à une famille virale (BYDV-GPV et BYDV-SGV ; King *et al.*, 2018). En Europe de l'Ouest l'espèce BYDV-PAV, est la plus fréquemment détectée devant BYDV-MAV et CYDV-RPV (Signoret et Maroquin, 1990).

Espèces vectrices

Environ 25 espèces de pucerons sont connues comme vecteurs des B/CYDV. Toutefois, l'efficacité de transmission dépend du couple espèce virale-espèce vectrice (Van den Eynde *et al.*, 2020). Par exemple, l'espèce de virus BYDV-PAV est principalement transmise par *R. padi*, *S. avenae* et *M. dirhodum*, l'espèce BYDV-MAV par *S. avenae* et *M. dirhodum*, et l'espèce CYDV-RPV par *R. padi* et *S. graminum* (Halbert et Voegtlin, 1995; Choudhury *et al.*, 2017; Van den Eynde *et al.*, 2020).

Espèces hôtes

Plus de 150 espèces végétales appartenant à la famille des Poacées sont connues comme pouvant être infectées par les virus B/CYDV, incluant les céréales cultivées tel que le maïs, le blé, l'orge, l'avoine, le seigle (D'Arcy, 1995; Lamptey *et al.*, 2003; Ali *et al.*, 2018). Les symptômes principaux associés à la JNO sont un nanisme des plantes atteintes, une décoloration des feuilles et une réduction de la croissance racinaire. Plusieurs composantes du rendement peuvent être atteintes tel que le nombre de talles, le nombre de grains par épi, le Poids de Mille Grains (PMG) (Smith et Sward, 1982), ce qui diminue le rendement en grain des plantes infectées. Ainsi, les pertes de rendements varient de 5% à 80% avec une diminution moyenne de 30% pour les champs infectés, accompagné d'une baisse de qualité du grain (Lister et Ranieri, 1995; Perry *et al.*, 2000; Dedryver *et al.*, 2010; Nancarrow *et al.*, 2021). Les symptômes associés à la JNO et leurs conséquences sur le rendement dépendent de plusieurs facteurs, tels que l'espèce et la variété de l'hôte végétale, son stade et son statut physiologique au moment de l'infection et de l'espèce de virus (e.g. PAV cause en générale des symptômes graves, MAV modérés et RPV faibles). Concernant l'espèce de plante hôte, les dégâts sur blé, orge et avoine sont les plus importants et répandus et ils sont particulièrement importants sur orge (Lister et Ranier, 1995). Concernant le stade de la plante hôte, les stades précoces de la levée à la fin du tallage, sont beaucoup plus sensibles que les stades plus avancés. Ainsi, Smith et Sward (1982) ont observé des pertes de rendements de 10-80% sur blé lors d'inoculation avant tallage et des pertes de 5-10% lors d'inoculation début montaison.

3.2.2. Phases et mode de vection du virus

Le processus de vection d'un virus de plante est divisé en quatre phases : la phase d'acquisition (AAP : Acquisition Access Period) où le puceron acquiert le virus depuis une plante infectée ; la phase de latence, pendant laquelle le puceron ayant acquis le virus (i.e. virulifère) est encore incapable de l'inoculer, et à la fin de laquelle il devient infectieux ; la phase d'inoculation (IAP : Inoculation Access Period) durant laquelle le puceron transmet le virus à une plante saine et la phase de rétention pendant laquelle le puceron reste infectieux.

Il existe quatre modes de transmission des virus de plante par les insectes piqueurs-suceurs, fondés sur la capacité du virus à traverser les barrières épithéliales du vecteur, appelés « circulant » ou « non circulant ». Pour les circulants, fondés sur la capacité du virus à se multiplier dans le vecteur, dits « multipliant » ou « non multipliant ». Enfin, pour les non-circulants, fondés sur le lieu de rétention du virus dans le vecteur, appelés « non persistant » (rétention au niveau du stylet) et « semi-persistant » (rétention au niveau du tube digestif antérieur) (Brault *et al.*, 2010). Les quatre modes de transmission des virus possèdent différentes caractéristiques (Table 1.2). Par exemple, les virus transmis de manière non-persistante sont transmis de manière non spécifique par de nombreuses espèces de pucerons, sont acquis très rapidement après de brèves piqûres d'essais et sont perdus rapidement dès que l'insecte se nourrit sur une nouvelle plante. À l'inverse, les virus persistants sont transmis spécifiquement par quelques espèces de pucerons, ont des durées d'acquisition et d'inoculation longues et le vecteur reste infectieux longtemps (Table 1.2).

Table 1.2. Caractéristiques des différents modes de vection d'agents phytopathogènes par des insectes. (Source : Herrbach *et al.*, 2013)

Pathogènes	Non circulants		Circulants	Circulants-multipliant
Mode	Non persistant	Semi-persistant	Persistant - non multipliant	Persistant-multipliant
Durées d'acquisition et d'inoculation	Secondes à minutes	Minutes à heures	Heures à jours	Heures à jours
Lieu d'acquisition	Épiderme	Phloème	Phloème	Phloème
Durée de latence	Aucune	Aucune	Heures à jours	Jours
Durée de rétention	Minutes à heures	Heures à jours	Plusieurs jours	Toute la vie
Passage transstadial	Non	Non	Oui	Oui
Multiplication	Non	Non	Non	Oui
Passage transovarier et/ou vénérien	Non	Non	Non	Possible pour certains pathogènes
Spécificité vectrice	Large	Étroite	Étroite	Étroite

Les B/CYDV sont transmis selon un mode circulant non multipliant par les pucerons des céréales (Gray et Gildow, 2003). Ce mode de transmission implique que les particules virales soient acquises par le puceron lors de l'ingestion de sève dans les tissus du phloème (Figure 1.5a). Concomitamment avec le bol alimentaire, les particules virales parcourent le tractus digestif jusqu'à atteindre l'intestin antérieur et/ou postérieur du vecteur (Figure 1.5a). En cas de compatibilité espèce de puceron-espèce de virus, les particules virales interagissent avec des récepteurs des cellules épithéliales de l'intestin, avant d'être internalisées par les cellules puis rejetées dans l'hémocoel (i.e. transcytose) (Figure 1.5a). Ainsi, les particules virales peuvent interagir avec diverses protéines présentes dans l'hémocoel du vecteur, ce qui pourrait assurer leur stabilité durant leur migration vers la lamelle basale des glandes salivaires (Brault *et al.*, 2010). Par un mécanisme de transcytose, les particules virales présentes dans

l'hémocoèle atteignent la lumière des glandes salivaires où elles seront mélangées avec la salive (Figure 1.5a). Ainsi, les particules virales pourront être inoculées pendant la salivation dans le tissu phloémien d'un nouvel hôte (Figure 1.5b). Aussi, le mode circulant non multipliant implique que le vecteur reste infectieux lors des mues et pour toute sa durée de vie. Toutefois, le virus n'est pas transmis à sa descendance et ne se multiplie pas à l'intérieur du vecteur (Table 1.2).

Une fois les particules virales des B/CYDV inoculées dans les tissus du phloème, une proportion d'entre elles initient leur réplication dans les cellules compagnes (Figure 1.5b) puis se propagent à plus grande distance aux racines puis aux feuilles grâce au flux du phloème (Waigmann *et al.*, 2004; Dedryver *et al.*, 2010) (Figure 1.5c). Les cellules compagnes réagissent à l'infection en produisant de la callose, bouchant progressivement les vaisseaux du phloème et entravant ainsi le passage de la sève. Cela entraîne une accumulation d'amidon dans les feuilles et une concentration en chlorophylle qui diminue, inhibant ainsi la photosynthèse (Dedryver *et al.*, 2010).

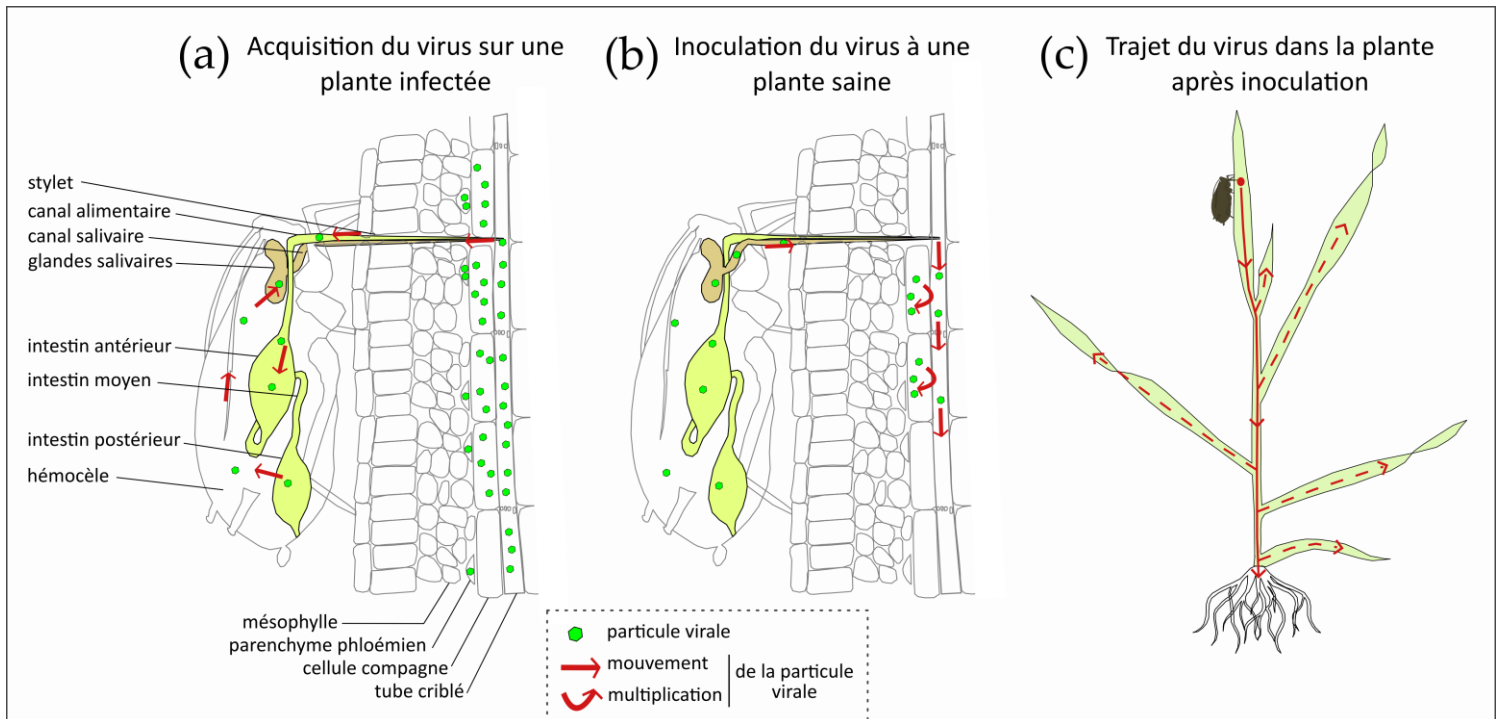


Figure 1.5. Schéma simplifié de l'acquisition et inoculation du virus par le puceron selon un mode circulant non multipliant et du trajet du virus dans la plante infectée. (Source graphique : Sarah Grauby).

3.2.3. Épidémiologie de la jaunisse nanisante de l'orge

Au cours de l'année, les pucerons des céréales sont mobiles dans le paysage agricole et alternent d'une plante hôte à une autre, à mesure que celles-ci terminent leur cycle de développement. Lors de ces événements de migration d'hôte à hôte, les pucerons transfèrent les virus dont ils sont les vecteurs. Entre deux cultures de céréales à paille, le maïs et les poacées sauvages représentent des « ponts » pour pucerons et virus. Le maïs est considéré comme un bon réservoir à pucerons mais une source de virus moins importante que les céréales à paille (Henry et Dedryver, 1989; 1991). Au contraire, les prairies sont un réservoir à pucerons beaucoup plus faible que le maïs, mais une source de virus plus importante, notamment grâce à leur statut pérenne et à l'accumulation d'infection virale au cours des années (Henry et Dedryver, 1991; Ingwell *et al.*, 2017).

Au champ, la prévalence d'une maladie repose sur un processus dynamique comportant une phase d'introduction du pathogène dans la parcelle depuis des réservoirs extérieurs (i.e. infections primaires) et sa propagation intra-parcellaire (i.e. infections secondaires). Pour la JNO, les pucerons ailés sont à l'origine de l'infection primaire de la maladie au champ et les pucerons aptères de l'infection secondaire, disséminant le virus à courte distance de plantes en plantes. L'importance d'un puceron comme vecteur de la maladie dépend de plusieurs facteurs tels que son espèce, sa plante hôte précédente, son moment d'arrivée sur la céréale, sa forme (sexuée ou parthénogénétique), son statut infectieux, sa survie et sa multiplication dans la culture (Plumb, 1995). L'épidémiologie de la JNO est également largement dépendante des conditions climatiques, que ce soit pour l'infection primaire, où les températures importantes favorisent le vol des ailés, mais aussi pour l'infection secondaire, où les températures élevées favorisent la reproduction, le mouvement des pucerons ainsi que la dispersion du virus (Huth et Lauenstein, 1991; Knaust et Poehling, 1996).

Céréale à paille semée en automne

En automne, les ailés provenant de poacées sauvages ou du maïs colonisent les jeunes semis de céréales d'hiver. La température minimale de vol des ailés est de 14-15°C. L'espèce majoritaire immigrant dans les cultures en automne est *R. padi* (Fabre, 2003), dont 7% en moyenne sont porteurs du virus, cette proportion pouvant aller jusqu'à 30% (Van den Eynde *et al.*, 2020). Plus une céréale est semée tôt en automne, plus les risques d'introduction d'ailés et d'infection sont importants (Plumb, 1992). Lors d'hivers doux, des populations peuvent continuer à se maintenir sur céréales, se reproduisant par parthénogénèse dès que les températures dépassent un seuil de -5°C (Bale, 1996; Brabec *et al.*, 2014). L'efficacité d'inoculation du virus par le puceron est aussi dépendante de la température, e.g. celle de BYDV-PAV par *R. padi* est de 33% à 6°C et 62% à 12°C (Lowles *et al.*, 1996). En sortie d'hiver, les céréales sont encore à des stades de développement précoces et sont toujours sensibles à l'infection par BYDV. Ainsi, sur les parcelles où les pucerons se sont maintenus par parthénogénèse tout l'hiver, les populations vont augmenter très rapidement avec l'augmentation des températures et continuent ainsi de disséminer le virus (Van den Eynde *et al.*, 2020).

Céréale à paille semée au printemps

Les céréales semées au printemps peuvent aussi être infectées par BYDV. Plus les céréales de printemps sont semées tôt, moins le risque d'infection est important (Jenkyn et Plumb, 1983). Les infections peuvent être sévères quand la levée des céréales, semées tardivement (avril/mai au lieu de février/mars) coïncide avec les vols des ailés ayant lieu au printemps.

4. Méthodes de lutte contre les pucerons des céréales

4.1. Lutte chimique

En agriculture conventionnelle, la protection phytosanitaire des céréales vis-à-vis des pucerons vecteurs de la JNO était principalement assurée par le traitement des semences avec des substances actives de la famille des néonicotinoïdes. L'effet systémique permettait une protection de la céréale de sa levée jusqu'à environ six semaines après son semis (Dewar, 2007). Dans les années 2000 en France, la part de semences certifiées traitées avec un produit néonicotinoïde était de 60% pour l'orge et 20% pour le blé, les pertes de rendement provoqués par la JNO étant inférieur pour le blé (Mc Namara *et al.*, 2020). L'interdiction des néonicotinoïdes au 1^{er} septembre 2018 par la loi pour la reconquête de la biodiversité, de la nature et des paysages a mis fin à leur usage.

Depuis l'interdiction des néonicotinoïdes, la lutte chimique contre les pucerons repose exclusivement sur l'utilisation de produits phytosanitaires en végétation à base de substance active de la famille des pyréthriinoïdes. L'efficacité de ce type de traitement est variable et dépend de nombreux facteurs tel que le positionnement du traitement par rapport au développement des populations de pucerons (l'efficacité étant maximale quand le traitement est réalisé avant l'arrivée des pucerons, la prédiction de l'arrivée étant très difficile), du nombre de traitements successifs réalisés (les feuilles émergent après un traitement n'étant pas protégées) et des conditions climatiques. De plus, des cas de populations *de S. avenae* résistantes aux pyréthriinoïdes ont été identifiés en Angleterre à partir de 2011, en Irlande et en Allemagne (Foster *et al.*, 2014).

4.2. Lutte agronomique

Ajuster la date de semis, en semant plus tard en automne et plus tôt au printemps, est une méthode culturale largement utilisée pour réduire le risque d'infection primaire de la JNO. En automne, le semis tardif de la céréale peut permettre d'éviter l'exposition des jeunes plantules aux vols de pucerons ailés. Cependant, l'efficacité de cette pratique est fortement diminuée en cas d'automne doux, où la fenêtre des vols de pucerons peut être allongée. Certaines années, un semis tardif peut être risqué, si les conditions climatiques se détériorent et empêchent le semis des céréales d'hiver (Plumb, 1995). Le travail du sol est une autre technique culturale utilisée, qui vise à détruire les repousses de céréales qui abritent le virus et ses pucerons vecteurs (Mc Namara *et al.*, 2020).

4.3. Lutte variétale

La résistance d'une plante face à un virus peut être séparé en deux catégories, i) la tolérance, lorsque les symptômes et les pertes de rendement sont réduits, sans que la multiplication du virus ne soit altérée et ii) la résistance, lorsque la multiplication et la propagation du virus dans la plante sont considérablement réduits (Jarošová *et al.*, 2016).

Malgré les milliers de ressources génétiques testées depuis plus de 50 ans, seules de rares sources de résistance/tolérance au complexe de la JNO ont été identifiées (Genty *et al.*, 2017). Chez l'orge, quatre gènes de tolérance ont été identifiés (*Ryd1*, *Ryd2*, *Ryd3* et *Ryd4*). Le gène *Ryd1* a un niveau de tolérance insuffisant et est rarement utilisé dans les programmes de sélection (Jarošová *et al.*, 2016). Le gène *Ryd2* a été découvert grâce à un screening extensif d'orge éthiopiens (Schaller *et al.*, 1964). Ce gène a été introduit dans de nombreuses variétés d'orge et est le principal gène de tolérance utilisé dans les programmes de sélection de l'orge (Kosová *et al.*, 2008). Ce gène a notamment permis de créer les variétés d'orge d'hiver tolérantes à la JNO : Naturel, Atenon, Amistar et Domino (inscrites au catalogue français en 1998, 2005, 2013 et 2014 respectivement) (Genty *et al.*, 2017). Seuls quelques milliers d'hectares sont cultivés avec ces variétés car elles ne conviennent pas à tous les débouchés (Genty *et al.*, 2017). Aucune variété portant le gène *Ryd3*, décrit en 2004, n'a été inscrite au catalogue à ce jour (Genty *et al.*, 2017).

Ainsi, la lutte génétique présente plusieurs limites : celle de la rareté des gènes de résistance/tolérance et la difficulté de produire des variétés commercialisables mais également, la limite de durabilité de ce trait. En effet, face à une plante hôte possédant ces gènes, des variants viraux capables de contourner la résistance/tolérance peuvent être sélectionnés (Genty *et al.*, 2017).

4.4. Lutte biologique par conservation

4.4.1. *Ennemis naturels des pucerons des céréales*

Les pucerons des céréales possèdent un cortège d'ennemis naturels possédant des degrés de spécificité variables. Les principaux prédateurs aphidiphages sont les coccinelles (Coleoptera : Coccinellidae), les chrysopes (Neuroptera : Chrysopidae) et les syrphes (Diptera : Syrphidae) (Müller et Godfray, 1999). La grande voracité des syrphes (stades larvaires) et des coccinelles (stades larvaires et adulte) (Freier et Triltsch, 1996; Tenhumberg, 1997; Ehsan *et al.*, 2020) et leur bonne synchronisation, dans le temps et dans l'espace, avec les pucerons des céréales font que ces ennemis naturels peuvent considérablement limiter le taux d'accroissement des populations de pucerons (Chambers et Adams, 1986; Poehling et Borgemeister, 1989; Hemptinne *et al.*, 1992; Tenhumberg et Poehling, 1995; Elliott *et al.*, 2000; Freier *et al.*, 2001). Les parasitoïdes quant à eux (Hymenoptera : Braconidae, Aphidiinae) sont les ennemis naturels des pucerons ayant le degré de spécificité le plus important.

Le cortège d'ennemis naturels généralistes des pucerons est composé de prédateurs tels que les carabes (Coleoptera : Carabidae), les araignées (Araneae) et les staphylins (Coleoptera : Staphylinidae). Les prédateurs généralistes peuvent contribuer de manière significative à réguler les populations de pucerons des céréales s'ils sont présents en densité suffisante en début d'infestation lorsque les pucerons colonisent les céréales (Chiverton, 1986; Edwards *et al.*, 1979; Wratten et Powell, 1991). Quand l'infestation de pucerons est plus avancée, la consommation de pucerons par les prédateurs généralistes est insuffisante pour diminuer fortement les populations de pucerons (Chiverton, 1986; Edwards *et al.*, 1979; Holland *et al.*, 1996; Wratten et Powell, 1991). Malgré leur consommation continue de pucerons, celle-ci reste faible en comparaison de celle des prédateurs spécialistes des pucerons (Sunderland *et al.*, 1987; Triltsch, 2001; Harwood *et al.*, 2005).

4.4.2. *Pratiques agricoles et régulation biologique des pucerons des céréales*

Dans le champ

Concernant la diversification végétale intra-spécifique, des études en laboratoire ont montré des effets de ce type de diversification pour réduire l'acceptance des pucerons des céréales pour leur plante hôte, pour certains couples de variétés (Pettersson *et al.*, 1999). Des études au champ ont confirmé ces résultats (Ninkovic *et al.*, 2002). Plusieurs auteurs ont également étudié l'effet de la diversification végétale inter-spécifique sur les pucerons des céréales. Helenius (1989) a observé des populations plus importantes (introduction des ailés et dissémination des aptères) des pucerons des céréales dans l'avoine associée à la féverole par rapport à l'avoine seul. Au contraire d'autres études associant du blé avec du colza ou du pois, ont observé des diminutions des populations de pucerons des céréales, parfois associées à une augmentation de leurs ennemis naturels foliaires et du parasitisme (Alarcón-Segura *et al.*, 2022; Lopes *et al.*, 2015; Wang *et al.*, 2009). Cependant toutes ces études ont suivi les populations de pucerons au printemps-été, même sur des cultures d'hiver. Ainsi, les effets de la diversification végétale intra-parcellaire sur les pucerons des céréales en début de cycle d'une céréale d'hiver, sont méconnus. Cette période est particulièrement importante puisque les pucerons sont vecteurs de virus et les infections en début de cycle peuvent engendrer des grosses pertes de rendements.

Les pratiques agricoles peuvent impacter l'abondance des ennemis naturels des pucerons des céréales. Par exemple, concernant le désherbage, des études ont observé que la distribution spatiale de différents prédateurs généralistes des pucerons est associée au degré de couverture des adventices (Holland, *et al.*, 1999; 2004). Le travail du sol, et particulièrement le labour, peut également impacter

la présence des ennemis généralistes des pucerons. Par exemple, Andersen (1999) a observé plus de carabes et de staphylins dans les modalités avec un faible travail du sol. D'autres études ont également montré que les ennemis naturels des pucerons des céréales peuvent être favorisés dans les systèmes céréaliers conduits en agriculture biologique par rapport à l'agriculture conventionnelle (Hasken et Poehling, 1994; Moreby *et al.*, 1994; Holland et Thomas, 1997).

Autour du champ

Les adultes parasitoïdes et syrphes ont besoin de ressources florales (pollen, nectar) comme source d'énergie et source de protéines pour leur reproduction (Chambers, 1988; Rivero et Casas 1999; Tenhumberg *et al.*, 2006). Les ressources florales, pouvant être assez rares dans les paysages agricoles intensifs (Heimpel et Jervis, 2005), l'incorporation de végétation non cultivée peut aider à soutenir la lutte biologique en augmentant leurs populations (Landis *et al.*, 2000). Par exemple, le semis de fleurs sauvages et de phacélie en bordure de parcelles de blé a permis d'augmenter les populations de syrphes adultes et le contrôle biologique des pucerons des céréales (Harwood *et al.*, 1992; Hickman et Wratten, 1996). Cependant, des travaux ont montré que les populations de parasitoïdes et leur activité de régulation déclinent dans la parcelle adjacente à mesure que la distance à la bande augmente (Tylianakis *et al.*, 2004; Lavandero *et al.*, 2005; Bianchi et Wäckers 2008).

Les bandes enherbées/fleuries autour des champs peuvent également représenter un habitat favorable ou un site d'hivernation pour les prédateurs généralistes des pucerons, leur fournissant un microclimat favorable ainsi que des proies alternatives. Ainsi, ces bandes peuvent entraîner des densités plus élevées de prédateurs généralistes dans les champs de céréales (Jmhasly et Nentwig, 1995; Lys *et al.*, 1994; Sotherton, 1984; 1985; Thomas *et al.*, 1992).

Partie 3. Contexte de l'étude

1. Contexte local

1.1. Production céréalière en France et en Auvergne-Rhône-Alpes

En France, les céréales les plus produites sont le blé tendre (32,7 Mt), le maïs (13,4 Mt) et l'orge (11,1 Mt) (Annexe 1). Concernant les surfaces semées en blé, 99% des surfaces en blé tendre et 94% des surfaces en blé dur sont des cultures d'hiver. Pour l'orge et l'avoine, respectivement 66% et 52% des surfaces semées sont des cultures d'hiver (Annexe 1). Cette prédominance des céréales à paille d'hiver dans l'assolement se retrouve en Europe centrale et méditerranéenne, le maïs étant la céréale préférentiellement semée au printemps dans cette zone pédoclimatique. Au contraire dans les zones plus au Nord, ce sont les céréales à paille qui sont en grande majorité semées au printemps, les conditions automnales et hivernales n'étant pas favorables à un semis d'automne (Plumb, 1995).

En Auvergne-Rhône-Alpes (AURA), les trois céréales les plus produites sont également le blé tendre (1,3 Mt), le maïs (1,2 Mt) et l'orge (0,4 Mt) (Annexe 2). La tendance française à ce que l'assolement soit plus tourné vers des céréales d'automne est exacerbée en Auvergne Rhône-Alpes. En effet, 99% des surfaces de blé tendre et 93% des surfaces en orge sont des cultures d'hiver (Figure 1.6a). La faible représentation des céréales à paille de printemps peut s'expliquer par différents facteurs dont un potentiel de rendement inférieur des céréales semées au printemps (Figure 1.6b) du fait d'un cycle plus court et décalé vers les périodes plus exposées au stress hydrique et thermique ; ou encore d'une utilisation des terres au printemps pour la culture de maïs au détriment des autres céréales de printemps.

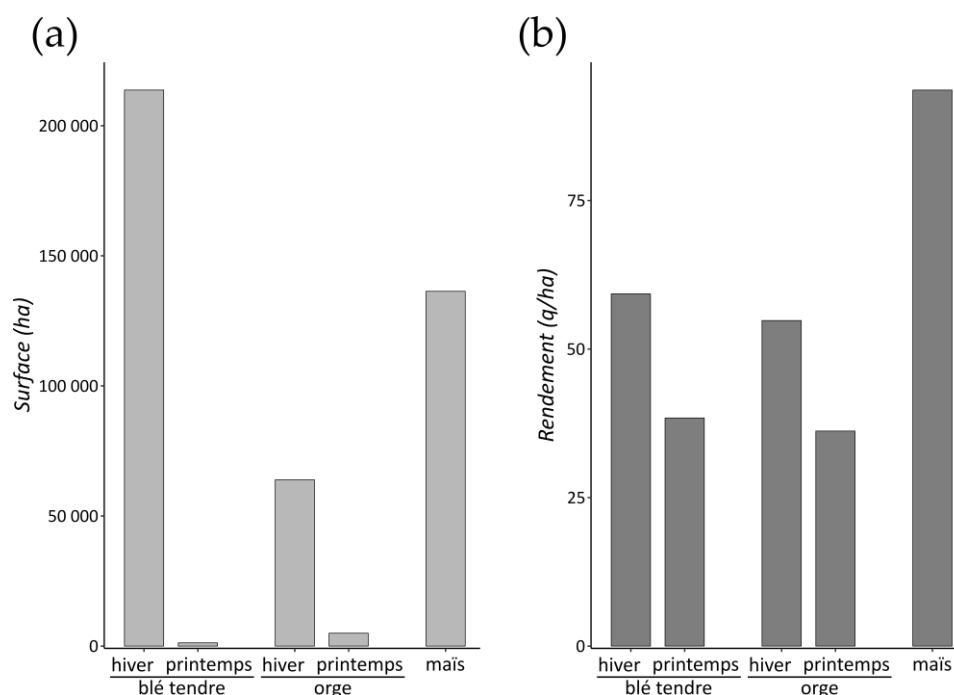


Figure 1.6. (a) Surface annuelle et (b) Rendement des trois principales cultures céréalières de Auvergne-Rhône-Alpes. (Source données : Agreste, moyennes de 2017 à 2021).

1.2. Développement de la filière brassicole

Ces dix dernières années, une explosion du nombre de brasseries artisanales et de microbrasseries a eu lieu en France. En effet, le nombre de microbrasseries, unité dont la production annuelle n'excède pas 1000 hectolitre, a été multiplié par six entre 2011 et 2021 passant de 373 à 2300 brasseries (Statista, 2023). Ces microbrasseries sont sorties des berceaux de production historiques du Nord et de l'Est de la France, s'implantant dans de nombreuses régions françaises. La région AURA est aujourd'hui la première région française en nombre de sites de productions (368 brasseries en 2020), même si la région Grand Est reste le leader en termes de volumes produits (Association des Brasseurs de France, s. d.).

En 2020, environ 30% des brasseries en AURA sont certifiées en agriculture biologique (Cluster bio Auvergne-Rhône-Alpes, 2020) et souhaitent s'approvisionner en ingrédients locaux certifiés en agriculture biologique (céréales, houblon). Concernant le malt (orge germée et séchée), ingrédient majeur pour produire de la bière, quatre malteries implantées en AURA fournissent du malt biologique et local aux brasseries. Cependant, une enquête récente montre que 80% des brasseries bio de la région s'approvisionnant déjà en malt bio régional souhaiteraient augmenter leur approvisionnement (Cluster bio Auvergne-Rhône-Alpes, 2021).

Ainsi, on observe ces dernières années une volonté des professionnels de la filière céréalière de développer les surfaces cultivées en orge brassicole bio en AURA. Cependant, la culture d'orge d'hiver, semée avant le blé d'hiver, est plus soumise aux vols de pucerons en automne et donc au risque d'infection primaire de la JNO. Or, en agriculture biologique, les leviers pour diminuer les populations de pucerons sont fortement limités. Le levier du semis tardif, particulièrement utilisé par les agriculteurs, voit son efficacité diminuer avec les automnes de plus en plus doux. Cette volonté de développement de production d'orge brassicole bio s'accompagne donc d'un questionnement sur l'existence d'alternatives ou de solutions complémentaires pour limiter le risque JNO. L'ISARA a donc choisi d'initier, en collaboration avec des professionnels de la filière céréalière, des essais chez des agriculteurs en agriculture biologique, pour tester de nouvelles méthodes agro-écologiques visant à réguler les populations de pucerons sur orge d'hiver et diminuer l'incidence de la maladie.

2. Contexte européen : le projet Ecostack

Ecotack est un projet H2020 européen, impliquant 13 pays, qui a débuté en septembre 2018 pour cinq ans (Figure 1.7). Le projet étudie des pratiques de protection des cultures qui permettent d'améliorer la durabilité des systèmes de production alimentaire en Europe. L'originalité de ce projet réside dans l'approche de « stacking » ou empilement de différents types ou niveau de biodiversité pour fournir une synergie dans les services bénéfiques rendus.



Figure 1.7. Pays impliqués dans le projet européen Ecotack. (Source : Ecotack, s.d.)

La thèse s'inscrit dans un des axes du projet Ecotack, qui étudie la pratique de diversification végétale intra-parcellaire pour diminuer les populations d'insectes ravageurs, pour renforcer le contrôle biologique par leurs ennemis naturels et ainsi améliorer les performances des cultures. Deux types de diversification ont été étudiés : la diversification intra-spécifique (mixture de variétés) et la diversification inter-spécifique (association d'espèces) ; ainsi que la combinaison de ces deux types de diversification (« stacking »). Des expérimentations au champ ont été réalisées dans plusieurs pays en culture de céréale (blé ou orge) et en culture de colza, avec comme ravageur respectif les pucerons des céréales et le méligèthe du colza. L'ISARA a participé aux expérimentations sur les pucerons des céréales.

En prélude aux expérimentations terrain, des essais en laboratoire ont été réalisés par la SLU (« Swedish University of Agricultural Sciences ») sur la diversification intra-spécifique et la communication plante-plante via les volatils suivant le protocole décrit dans Ninkovic *et al.* (2002). Ces essais visaient à déterminer, parmi une sélection de variétés de céréales envoyée par chaque pays expérimentateur, le couple de variété le plus prometteur en termes de diminution de l'acceptance des pucerons pour la céréale hôte. Chaque pays a ensuite testé au champ, en micro-parcelle ou en grande parcelle d'essai (conditions agriculteur) les différents types de diversification végétale (dont la diversification intra-spécifique avec le couple de variétés sélectionné pour le pays). Ces essais ont été conduits sur céréale à paille (blé ou orge) soit en culture d'hiver (pays du Sud e.g. Espagne, France, Serbie) soit en culture de printemps (pays du Nord e.g. Suède, Angleterre). Les protocoles de suivis sur le terrain (pucerons, ennemis naturels, rendement) ont été discutés au sein du consortium en vue de les homogénéiser. Les suivis sur le terrain ont été réalisés au printemps/été, pour avoir une période commune à tous les pays partenaires.

Partie 4. Objectifs du travail de thèse et organisation du manuscrit

Problématique

Ainsi, la problématique posée dans le cadre de la thèse est la suivante :

La diversification végétale intra-parcellaire permet-elle une régulation biologique des pucerons des céréales ?

Objectifs

Les deux principaux objectifs de la thèse sont les suivants :

- 1) Tester le potentiel de la diversification intra-parcellaire en automne-hiver pour réguler les populations de pucerons des céréales, vecteurs des virus BYDV, particulièrement préjudiciables pour la céréale d'hiver.
- 2) Tester le potentiel de la diversification intra-parcellaire au printemps pour réguler les populations de pucerons des céréales, pouvant causer des dégâts directs à la céréale.

Les types de diversification végétale testés

Plusieurs types de diversification végétale intra-parcellaire ont été testés dans cette thèse :

- i) la diversification intra-spécifique : avec un mix de deux variétés d'orge brassicole d'hiver.
- ii) la diversification inter-spécifique : avec l'association de l'orge à du trèfle. Le trèfle a été choisi dans une logique de couvert « multi-services ». Les deux cultures ont été associées sans arrangement spatial particulier (« mixed intercropping ») et semées en même temps (« simultaneous intercropping »).
- iii) les deux types de diversification combinés, dans une logique de « stacking ».

Hypothèses

Compte tenu du peu de littérature disponible sur l'effet de la diversification végétale intra-parcellaire en automne-hiver, des hypothèses communes aux deux périodes ont été formulées :

- la diversification intra-spécifique permet une diminution des populations de pucerons des céréales.
- la diversification inter-spécifique permet une diminution des populations de pucerons des céréales (mécanismes bottom-up et top-down) ainsi qu'une augmentation des populations de leurs ennemis naturels (par la provision d'habitat et de sources de nourriture pour les prédateurs du sol et par la provision d'hôtes/proies alternatives et de source de nourriture sucrée pour les ennemis naturels foliaires).
- la combinaison des deux types de diversification permet une diminution encore plus importante des populations de pucerons des céréales.

Les types d'expérimentation réalisés

Pour répondre à la problématique de thèse, et plus spécifiquement à ses deux objectifs, plusieurs expérimentations ont été conduites.

- Dans un dispositif multi-plantes en conditions contrôlées, la diversification inter-spécifique a été testée afin de déterminer son potentiel pour i) diminuer les populations de *R. padi* et la prévalence de la JNO, ii) altérer la distribution spatiale des pucerons et du virus. Le second objectif a été d'étudier si l'effet de la plante compagne diffère selon le morphe (ailé ou aptère) du puceron fondateur. Cette expérimentation est présentée dans le **Chapitre 3**.
- Dans un essai au champ en conditions « agriculteur » conduit sur les campagnes 2019-2020 et 2020-2021, les deux types de diversification et leur combinaison ont été testés, afin d'étudier leur potentiel à plusieurs périodes clés du cycle d'une céréale d'hiver pour :
 - **En automne-hiver**, diminuer les populations de pucerons des céréales (introduction des ailés et dissémination des aptères), diminuer la prévalence de la JNO et attirer les ennemis naturels des pucerons (terrestres et aériens). Cette expérimentation est présentée dans le **Chapitre 4**.
 - **Au printemps**, diminuer les populations de pucerons des céréales et attirer les ennemis naturels (terrestres et aériens) des pucerons. Cette expérimentation est présentée dans le **Chapitre 5**.
 - **À la récolte**, augmenter les performances agronomiques des cultures. Cette expérimentation est présentée dans le **Chapitre 6**.

NB : Cette thèse est rédigée sous forme d'articles scientifiques. Ainsi les chapitres 3 à 6 représentent 4 articles. Quand le contenu d'un chapitre est trop important pour être contenu dans un seul article, il a été choisi qu'il soit séparé en un article suivi d'une partie résultats supplémentaires. C'est le cas des chapitres 4 et 5. De plus, une synthèse des résultats sous forme d'une figure est proposée à la fin des chapitres 4 et 5.

CHAPITRE 2

MATERIELS ET METHODES



- C. Grauby.

Aquarelle, trèfle incarnat
Trifolium incarnatum
Christine Grauby

Chapitre 2. Matériels et Méthodes

Two different type of research were conducted in order to assess the potential of plant diversification within the field to reduce cereal aphid populations. A field experiment on different farmers' fields was implemented to investigate the effect of plant diversification (intra-, inter-specific and both combined) at two key periods in the barley growing cycle where cereal aphids can cause damage to the barley (chapters 4, 5, 6). To further investigate one aspect of the field experiment (aphids as vector-borne virus in autumn-winter), a laboratory experiment was conducted. It studied the effect of inter-specific plant diversification on *Rhopalosiphum padi* and on BYDV-PAV virus under controlled conditions (chapter 3 of the manuscript).

1. Laboratory experiment

A laboratory experiment was carried out from March to May in 2021 (collaboration at PHIM, Montpellier). In arenas (i.e. sets of 36 barley plants) barley was intercropped with clover at two different sowing densities, or grown alone (Figure 2.1a, Figure 2.2). A viruliferous *R. padi* was deposited (with an alate or wingless morph). After 13 days the aphids on plants were counted and killed. After 21 days, the plants were tested by ELISA to detect the presence of BYDV-PAV (Figure 2.1b). Chapter 3 of the manuscript focuses on this experiment and presents it with more detail.

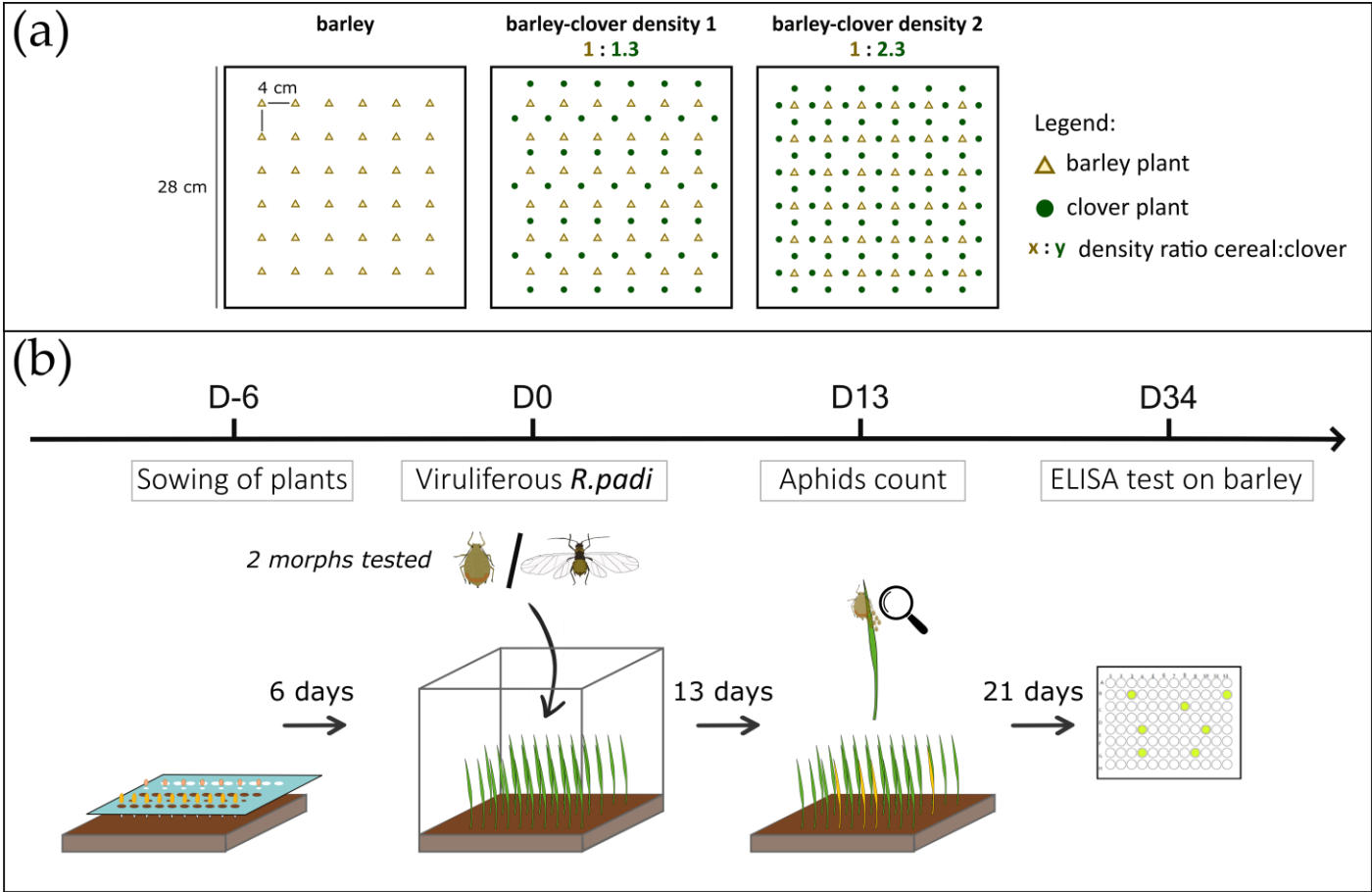


Figure 2.1. (a) Design and (b) protocol over time of the laboratory experiment.



Figure 2.2. Three treatments tested in the laboratory experiment. Barley alone (left), barley intercropped with clover at the density 1 (middle) and barley intercropped with clover at the density 2 (right). Arenas at day 11.

2. Field experiments

2.1. On-farm research sites

Field experiments were implemented on commercial farms under organic agriculture in the Rhône-Alpes region (South-East of France) over two consecutive winter barley growing seasons (2019-2020 and 2020-2021) (Figure 2.3). In total, the on-farm study was implemented on 10 fields (5 fields per year of monitoring), involving 8 different farmers. Fields were located within a 50 km radius around the city of Lyon. The field experiments were set up on relatively small fields (on average 4 ha and varying from 1 ha to 9 ha).

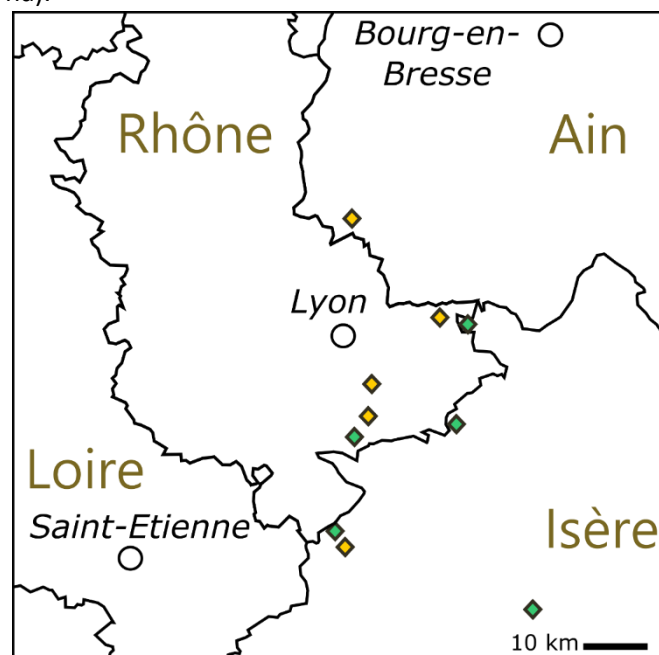


Figure 2.3. Location of the experimental sites. The field experiment was implemented on 5 farmers' organic fields in 2019 (◆) and 5 fields in 2020 (◇), located in the Ain, Rhône and Isère departments of the Rhône-Alpes region.

2.2. Experimental design

Prior to the implementation of the field experiment, and in the frame of a larger European research project, a lab screening research was carried out by one of the project partners, following the protocol described in Ninkovic *et al.* (2002). It was conducted in order to determine the best possible pairwise combinations among three French candidate barley varieties to reduce aphid acceptance

(Amistar, Margaux, Salamandre). Amistar and Margaux together was the best combination and allowed a 23% reduction of *Rhopalosiphum padi* acceptance (Ninkovic 2019, not published). Thus, these two winter barley (*Hordeum vulgare* L.) varieties were used, both varieties being tolerant to BYDV, and allowing an early sowing of field experiments in autumn without risking major yield losses for farmers.

The undersown clover species mixture was composed of *Trifolium alexandrinum* (*T. alexandrinum*), *Trifolium incarnatum* (*T. incarnatum*), and *Trifolium repens* (*T. repens*) species, in order to provide a good soil cover of the sowed areas during the barley growing season (Figure 2.4).



Figure 2.4. Clover species used in mixture in the field experiment. *T. incarnatum* (left), *T. alexandrinum* (middle) and *T. repens* (right). Plants sampled in April 2020 in one of the trials.

Each field experiment was composed of six different treatments: three treatments with barley alone, i.e., barley variety Amistar, barley variety Margaux, and a mixture of the two varieties; and three treatments with barley associated with clover, i.e., barley variety Amistar/clover, barley variety Margaux/clover and a barley variety mixture/clover. Each treatment was seeded on a plot of 24 m x 50 m (Figure 2.5a). The winter barley was sown at 150 kg/ha (355 seeds/m²), with a substitutive design for the barley mixture. The clover cover was sown at 13 kg/ha using a mixture at the rate of 5 kg/ha for *T. alexandrinum* and *T. incarnatum* and of 3 kg/ha for *T. repens*. Winter barley and clover were sown simultaneously in October. Each trial was sown on a farmer's field (Figure 2.5b), and the rest of the field was sown with barley (variety Amistar). Field experiments were not treated with any pesticides and weeds were mechanically controlled (one harrowing pass between November and March) except in the clover growing areas. Fields were fertilised once (in January/February) with organic fertilisers.

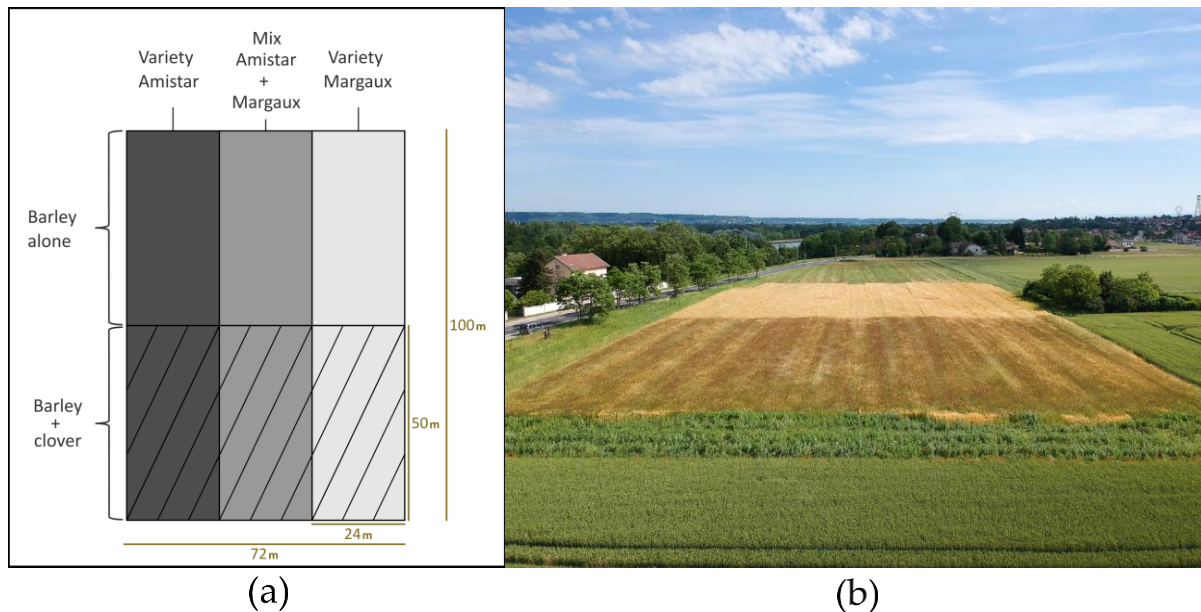


Figure 2.5. Design of the field experiment. Two factors are tested in six treatments: the "variety" factor (Amistar, Margaux and Mixture) and the "cover crop" factor (with or without clover); **(b)** Drone photo of one of the trials in June 2021. The treatments with clover are on the lower part of the picture.

2.3. Monitoring methods

Two main monitoring periods took place during the barley growing season: in autumn-winter and in spring. During these two periods of the cycle, aphids can cause damages to the crop, either indirectly in autumn-winter via the transmission of viruses or directly in the spring via sap feeding (Figure 2.6). In autumn-winter, in the different treatments of the trial, alate aphids (able to introduce BYDV into the field), aphids on plants (able to spread BYDV within the field) as well as the occurrence of BYDV on plants were monitored. Natural enemies of aphids (foliar or terrestrial) were also recorded. In spring, aphids on plants and their natural enemies were monitored (Figure 2.6).

NB: For all types of monitoring (aphids, ground-dwelling arthropods, *etc.*), when all the fields are sampled on the same day, we will refer in the manuscript to a "sampling date". When all the fields are sampled on a few successive days (which is frequently the case, being difficult to monitor all the fields in the same day), the term "sampling period" will be used.

NB2: Two years of monitoring were thus conducted during the winter barley growing cycle: in 2019-2020 and 2020-2021. For convenience, we will refer in the manuscript to winters 2019 and 2020 (when in reality the winters overlap two years) and springs 2020 and 2021.

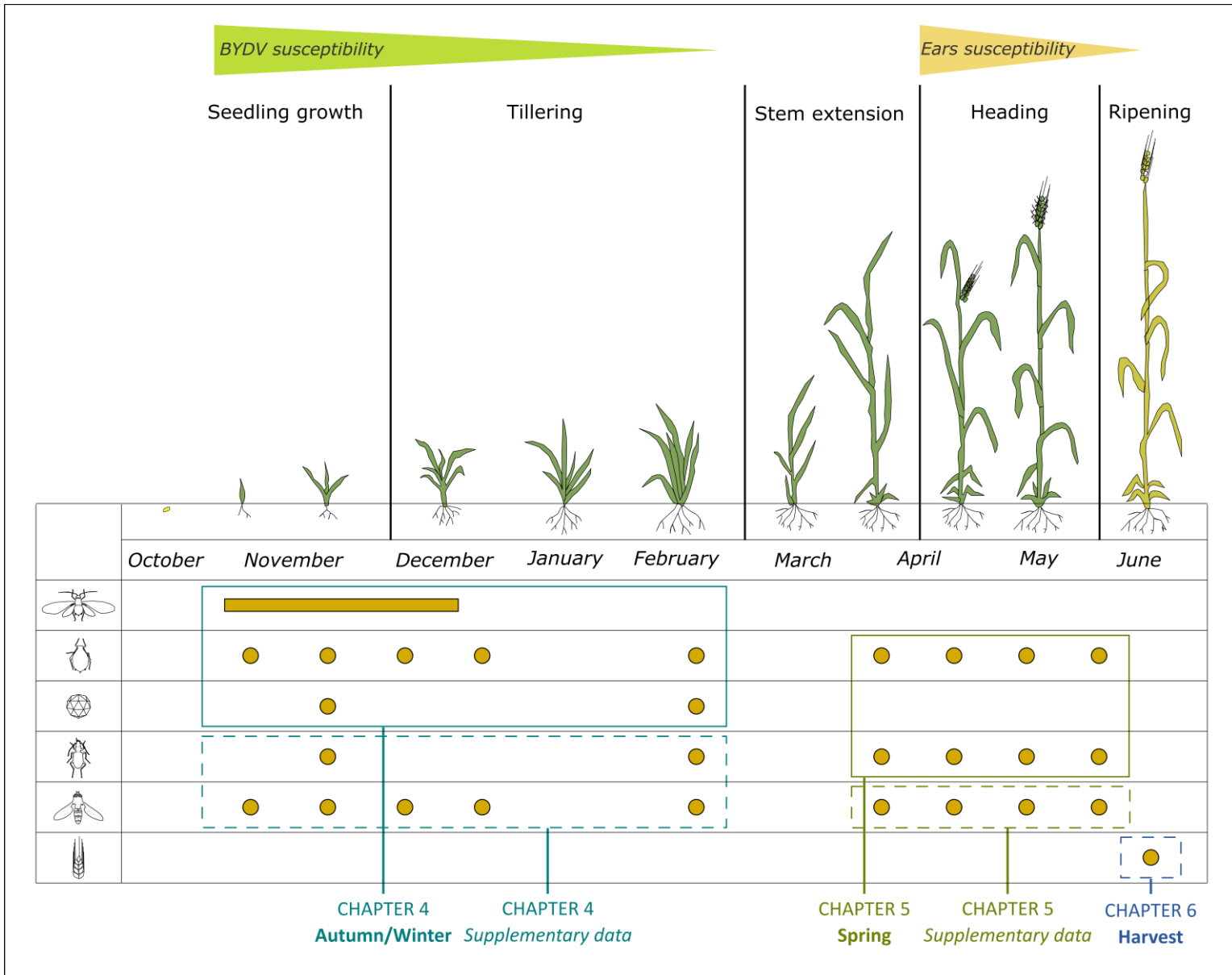








Figure 2.6. Winter barley development cycle, sensitive stages to cereal aphids (indirect or direct damages) and different barley and insect monitoring conducted during the field experiment. Monitoring of: alate aphids

(); aphids on barley plants (); BYDV in plant tissue (); ground-dwelling arthropods (); aphid

foliar enemies (); barley yield and its components (.

For each type of monitoring, each item (●) represents one sampling period (i.e., a few dates, on which all the sites of the experiment were monitored). The item (■) represents the monitoring period of alate aphids which was continuous.

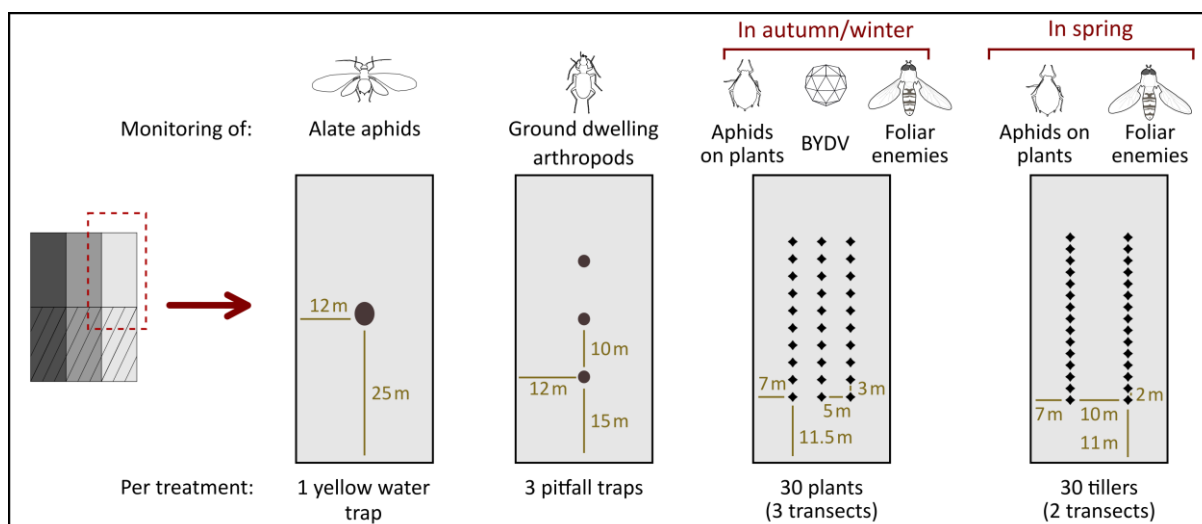


Figure 2.7. Monitoring protocols implemented in the field experiment.

2.3.1. Aphids monitoring

Alate aphids were monitored using yellow water traps, with one trap placed in the centre of each treatment (Figure 2.7, Figure 2.8a). Traps contained water supplemented with few drops of odourless and colourless soap. This aphid monitoring started two weeks after sowing and lasted until mid-December (Figure 2.6). Caught insects were collected every 3-5 days in 2019 (due to technical reasons and field planning) and every 7 days in 2020.

In autumn-winter, aphids within the field were monitored on 30 barley plants sampled along three parallel transects per treatment. Per transect, aphids were counted on 10 plants, with a distance of 3 m between two plants. Within each treatment plot, the three transects were spaced 5 m apart, and 7 m from the edges of the plot (Figure 2.7). Each sampled plant was carefully cut and the number of aphids on it was recorded (Figure 2.8b). For each aphid individual, species, development stage (nymph or adult) and adult morph (alate or wingless) were recorded. Aphids were monitored only under good weather conditions (no rain, little or no wind and after morning dew). This aphid monitoring was conducted over four sampling periods from the emergence of the cereal, early November, to mid-December. An end-winter sampling period was done at the end of February (Figure 2.6). In spring, aphids within the field were monitored following the same method as in autumn-winter with some minor differences: 30 tillers were sampled (instead of 30 plants), distributed over two transects (instead of 3), the distance between two sampled tillers being 2 m (instead of 3 m) (Figure 2.7). This aphid monitoring was carried out at four stages of the cereal development in spring (Figure 2.6): booting (end-March), heading (end-April), flowering (early-May) and milk development (May).

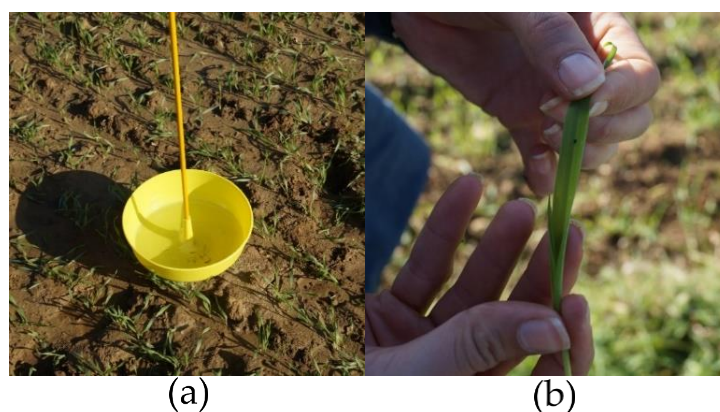


Figure 2.8. Protocols used for aphid monitoring. Monitoring of (a) alate aphids with yellow pan traps; (b) aphids on plants by visual counting.

2.3.2. BYDV monitoring

In order to conduct BYDV occurrence analysis, barley leaf sampling was carried out on 30 plants per treatment (the same as aphids counting) (Figure 2.7). On each plant, the last two developed leaves were collected and packaged together in a plastic bag. The bags were stored at -20°C, until the presence of the virus in each sample was determined by serological analysis (ELISA). Two barley leaf sampling periods were performed, mid-November, end of February. The protocol followed for the ELISA analysis is described in the chapter 4.

2.3.3. Aphid natural enemies monitoring

Foliar enemies of aphids were counted during the aphid monitoring, on the same plants (Figure 2.7). For the foliar predators, their development stage was recorded (egg, larva, pupa or adult). For the parasitoids, mummy abundance (i.e. last parasitoid larval instar) was monitored, as they are easily countable in the field. For each mummy, it was recorded if the parasitoid had emerged through the observation of emergence hole (Figure 2.9a). Parasitism rate was calculated as the ratio of mummies to total aphids (including mummies). Foliar enemies were monitored during the two key periods (autumn-winter and spring) (Figure 2.6). In autumn-winter, after counting, the mummies were collected and transferred to the laboratory. The emergence of parasitoids was regularly monitored.

Ground-dwelling arthropods were monitored using three pitfall traps placed in the central line of each treatment. Traps were placed 10 m from each other and 15 m away from the plot edges (Figure 2.7). Pitfall traps (Ø 10 cm) were half-filled with a 30% propylene glycol solution supplemented with a drop of odourless soap and covered by a plastic plate (Ø 17 cm) to protect the samples (rain, bycatch) (Figure 2.9b). The traps were opened for 48 hours. Ground beetles and spiders were identified at the species and at the family level, respectively, while rove beetles were counted without further identification. Two trapping periods were set up in autumn-winter (mid-November and end-February). Four trapping periods were set up in spring, at the same time as cereal aphid monitoring, from end-March to end-May (Figure 2.6).

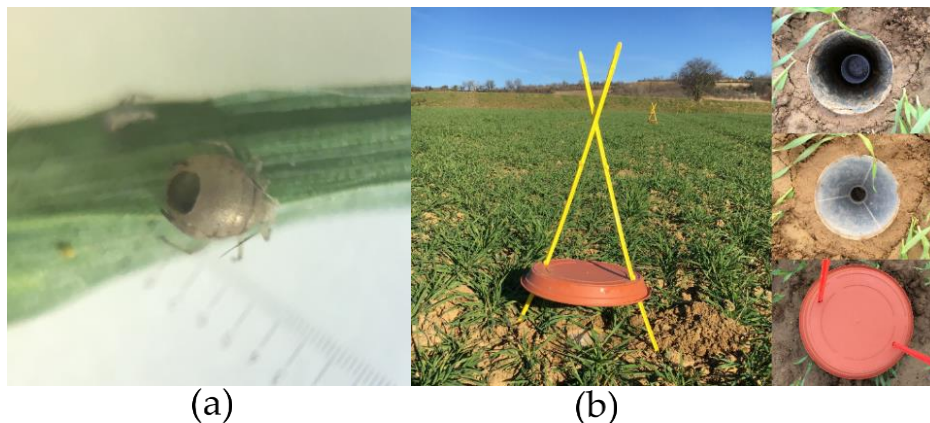


Figure 2.9. Protocols used for aphid natural enemies monitoring. Monitoring of (a) aphid foliar enemies on plants by visual counting; (b) ground-dwelling arthropods with pitfall traps.

2.3.4. Crop monitoring

2.3.4.1. Crop development

The development of the different crops was monitored in 0.25 m² quadrats (Figure 2.10a), randomly replicated three times per treatment. In each quadrat, the percentage of barley and clover soil cover was recorded by visual estimations. Then, the average height of each crop was measured (barley and the three clover species) and their growing stage was recorded. In autumn, crop development was assessed at two sampling periods in 2019 (end of November and mid-December) and weekly in 2020 from November to mid-December and one last sampling period was held at the end of February. In spring, crop development was assessed at four sampling periods at the same time as cereal aphid and ground-dwelling arthropods monitoring.

2.3.4.2. Biomass and yield

At barley harvest, the above-ground biomass produced by the different crops, the barley grain yield and different yield components were monitored (Figure 2.6). Six samples per treatment were collected using 0.25 m² quadrats, in which the above-ground biomass (barley, clover and weeds) was hand-harvested and sorted (Figure 2.10b). The collected samples were oven-dried for 48 hours at 60°C and then weighed. Per barley sample, straw and spikes were weighed separately and the number of spikes was determined. Then the spikes were threshed and the grains were weighed. A sub-sample of 350 grains per sample was taken and weighed, in order to calculate the 1000-grain weight.

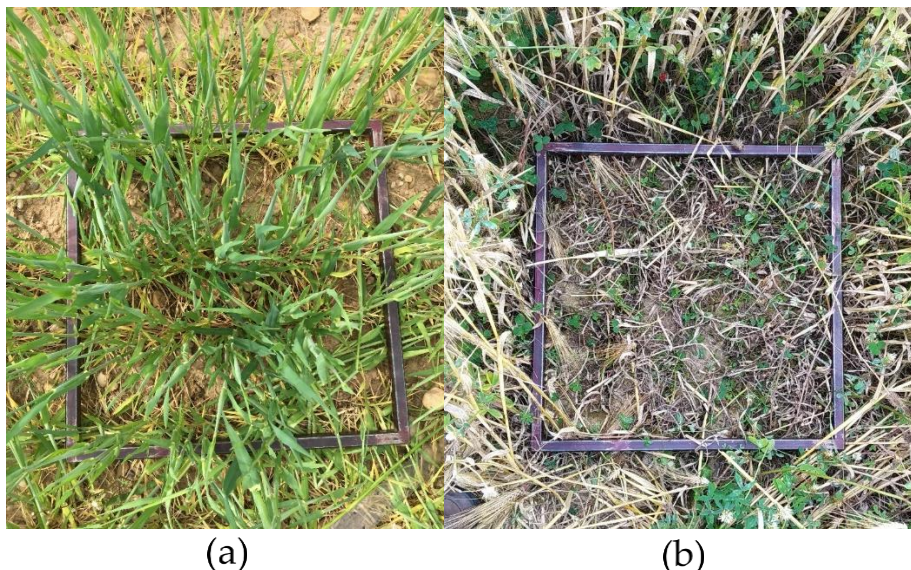


Figure 2.10. Protocols used for crop monitoring. Monitoring of (a) crop development by visual estimation; (b) biomass, yield and its components by above-ground biomass harvest.

2.3.5. Temperature recording

On each field experiment, a Tiny-tag data logger (Tinytag plus 2, Gemini Data Loggers, Chichester, UK) was placed to record temperatures (maximum and minimum) every 30 minutes. Data loggers were kept during the whole experiment period (from barley sowing to harvest).

CHAPITRE 3

EFFET DE LA DIVERSIFICATION VEGETALE INTER-SPECIFIQUE
SUR LES POPULATIONS DE *RHOPALOSIPHUM PADI* ET LA
DISPERSION DU VIRUS RESPONSABLE DE LA JAUNISSE
NANISANTE DE L'ORGE



Aquarelle, *Rhopalosiphum padi* et ses larves
Christine Grauby

Chapitre 3. Effet de la diversification végétale inter-spécifique sur les populations de *Rhopalosiphum padi* et la dispersion du virus responsable de la jaunisse nanisante de l'orge

Can mixed intercropping protect cereals from aphid-borne viruses? An experimental approach.

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Grauby, S.; Ferrer, A.; Tolon, V.; Roume, A.; Wezel, A.; Jacquot, E. Can Mixed Intercropping Protect Cereals from Aphid-Borne Viruses? An Experimental Approach. *Insects* 2022, 13, 521. <https://doi.org/10.3390/insects13060521>

Keywords: BYDV-PAV; *Rhopalosiphum padi*; morph; arena experiment; companion plant; clover; barley

Abstract: Intercropping, i.e., association of two or more species, is promising to reduce insect populations in fields. The cereal aphid *Rhopalosiphum padi*, a vector of the Barley yellow dwarf virus PAV (BYDV-PAV), represents a major threat for cereal grain production. In this study, we tested the potential of winter barley intercropped with clover to reduce the size of *R. padi* populations and to lower the BYDV-PAV incidence in fields. We used arenas (i.e. sets of 36 barley plants) intercropped with or without clover plants (at different sown densities). In each arena, a single viruliferous founder, *R. padi*, (with an alate or a wingless morph) was deposited to introduce aphids and viruses in the experiment. Thirteen days later, the number of aphids in the arena, the percentage of plants hosting aphids and the infection rates were monitored. Data produced through this experimental design showed that clover alters the distribution of the aphid progeny (lower aphid spread) produced by an alate founder morph. Moreover, clover reduces the size of aphid populations produced by a wingless founder morph. However, despite the effects of clover on biological parameters of *R. padi*, the presence of clover in barley arena did not modify BYDV infections, suggesting complex mechanisms between partners of the BYDV pathosystem for plant-to-plant virus spread.

1. Introduction

Yellow dwarf disease (YDD) is one of the most important viral diseases in cereal crops worldwide, causing dwarfing, leaf discoloration, and yield losses up to 80% (Perry *et al.*, 2000; Nancarrow *et al.*, 2021). The disease is caused by a complex of ten virus species, transmitted in a persistent non-propagative manner by aphids (Gray and Gildow, 2003). At least twenty-five aphid species are involved in the epidemiology of YDD (Halbert and Voegtlin, 1995), and each of the ten virus species has its primary and secondary vectors species. In France, the bird cherry-oat aphid *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) is considered to be the main vector of YDD. Indeed, it is able to efficiently transmit BYDV-PAV, the most prevalent and damaging virus species reported in French cereal fields so far (Lister and Ranieri, 1995). As many aphid species, *R. padi* exhibit wing dimorphism at different stages of its life cycle. In autumn, primary infections occur when viruliferous alate *R. padi*, whose flight

threshold is 10°C (Wiktelius, 1981), introduces the virus into newly sown winter cereal fields. Then, when temperatures are above 5°C, wingless individuals produced by parthenogenesis move from plant to plant and spread the virus within the field (Elliott and Kieckhefer, 1989). This step of the epidemical cycle corresponds to the secondary infections. When cereals are at an early development stage, they are highly susceptible to YDD. Thus, yield losses induced by YDD in winter cereals are mainly due to autumn infections (Carrigan *et al.*, 1981; Leclercq-Le Quillec *et al.*, 1995). As winter approaches, sexual alate individuals are produced and meet on the primary host of *R. padi*, i.e., *Prunus padus*, a small tree non-host for YDD. They form eggs which are more resistant to cold, and that will be the source of new spring populations (Brabec *et al.*, 2014). However, in regions with mild winters, survival in egg form for aphids is less advantageous and reproduction in an asexual form (parthenogenesis; i.e., anholocyclic populations) continues throughout the cereal growing season. Global warming in temperate regions may impact the epidemiological cycle of YDD through the increase of anholocyclic aphid populations (Van den Eynde *et al.*, 2020), the presence of aphids in cereal fields over a longer period, and the increase of virus spread. Thus, the damage related to YDD is expected to increase in the coming years.

Control methods used by farmers against YDD are limited. Cultural methods, such as late sowing and removal of volunteers, could constitute good options to limit the incidence of YDD on winter cereals (Poehling *et al.*, 2007). However, mild autumns, which are increasingly frequent in a context of global warming, decrease the efficiency of late sowing. In conventional farming, insecticides were mainly used to control YDD. However, since the ban of neonicotinoids at the end of 2018, only pyrethroid-based foliage treatments remain with variable efficiencies. Thus, in the context of (i) the reduction of the use of plant protection products in cropping systems and (ii) the global warming, it is necessary to find new methods or practices to reduce aphid populations and YDD incidence.

The agroecological transition proposes new practices, relying more on natural processes and less on synthetic inputs. Agroecosystem diversification is one of the two pillars of this transition (Nicholls and Altieri, 2004) and proposes to insert practices based on plant diversification at different scales. Among these practices, plant species association (i.e. intercropping) has been shown to bring many benefits for disease and pest managements. Concerning aphids, numerous studies show a decrease in their populations in intercropping systems compared to monocultures (Smith, 1969; Smith, 1976; Horn, 1981; Costello and Altieri, 1995; Hooks *et al.*, 1998; Showler and Greenberg, 2003). This biological control can be explained by top-down (indirect effect of the non-host plant on aphids via natural enemies) and bottom-up (direct effect of the non-host plant on aphids) mechanisms. In the bottom-up mechanisms, the non-host plant may interfere at different stages of the selection of host plants by aphid. Firstly, the non-host plant can interfere with different stimuli (olfactory (Nottingham *et al.*, 1991; Nottingham and Hardie, 1993), visual or physical (Perrin and Phillips, 1978)), that the aphid uses to detect its host plant. Then, if an aphid inadvertently arrives/lands on a non-host plant, it will start to make brief shallow test probes with its stylets (Powell *et al.*, 2006) to evaluate the quality and the host/non-host status of the plant. The aphid can thus waste time testing a plant on which it will not feed and reproduce. Furthermore, once the host plant is found, the aphid can spend less time on it in an intercropping situation, as it has been shown in a recent study (up to 40% reduction of time spent) (Mansion-Vaquíé *et al.*, 2020). The main consequences of a shorter period spent on host plants by aphids are the production (i) of fewer offspring and (ii) milder direct (through sap removal) and/or indirect (through aphid-mediated transmission of viruses) damages. Thus, at field scale, one of the expected consequences of intercropping is a decrease of aphid populations and a lower aphid-mediated transmission of viruses. However, the impact of intercropping on the epidemiology of viral

diseases has been poorly studied. Indeed, authors generally suggest that the decrease in aphid populations in an intercropping area probably leads to a decrease in the disease incidence. Hooks and Fereres (2006) reviewed the effect of intercropping on viruses transmitted by aphids in a non-persistent manner and propose four potential mechanisms explaining the reduction of disease incidence. Concerning viruses transmitted in a persistent manner, which is the case for BYDV-PAV, studies on the impact of intercropping on viral epidemiology are lacking.

In this study, we chose clover as a non-host plant to disrupt *R. padi* and BYDV-PAV colonisation of barley fields. This companion plant is already widely used in association with crops by organic farmers for many other services like nitrogen supply to the following crop, weeds control, and soil cover after the cereal harvest (Bergkvist *et al.*, 2011; Amossé, 2013). To accurately describe the impact of clover on epidemiological parameters involved in the introduction and spread of YDD in fields, lab experiments with pots containing barley plants, in the presence or absence of clover, were used and monitored over several weeks. Moreover, two clover densities were tested. The following hypotheses were formulated:

- 1) The *Rhopalosiphum padi* total abundance in a multiple plants system is decreased in the presence of clover.
- 2) The BYDV-PAV incidence in barley is reduced in the presence of clover in arena.
- 3) The spatial distributions of aphids and virus in arena are modified in the presence of clover.
- 4) The effects of clover are more important for wingless founder aphids than for alate founder aphids.

2. Materials and methods

2.1. Plants, insects and virus

Barley seeds (cv. Etincel, [Sécobra France]) were sown in N2 soil (Neuhaus® Huminsubstrat N2, klasmann Deilmann, Geeste, Germany) in square pots (L × W × H: 28 × 28 × 7 cm) in the presence or the absence of clover seeds, *Trifolium incarnatum* (cv. Viterbo). A perforated plexiglass seeding plate was designed to sow the different seeds (barley and clover) at specific locations in pots (Figure 3.1). Pots contain 36 barley seeds (6 rows × 6 lines) with a 4 cm distance between each seed. Two clover densities were tested because the efficiency of the barley/clover association could be dependent on the density of clovers in the arena. Square pots containing only barley seeds are noted as b in the manuscript. The bc1 and bc2 square pots referred to pots containing barley plantlets associated to 45 and 84 clovers, respectively (Figure 3.1). Barley and clover seeds were sown to a depth of 2 cm and 1 cm, respectively. Six days after sowing, the germination of the barley seeds was checked, and missing plantlets were replaced by extra plantlets produced under the same conditions. A similar checking and replacement procedure was applied to clover plants but with an expected germination threshold of 80% per pot, leading to a minimum of 36 clovers in bc1 and 68 clovers in bc2. Pots were maintained in a growth chamber (day/night: 16 h/8 h; 24°C) from sowing to the end of aphid exposure.

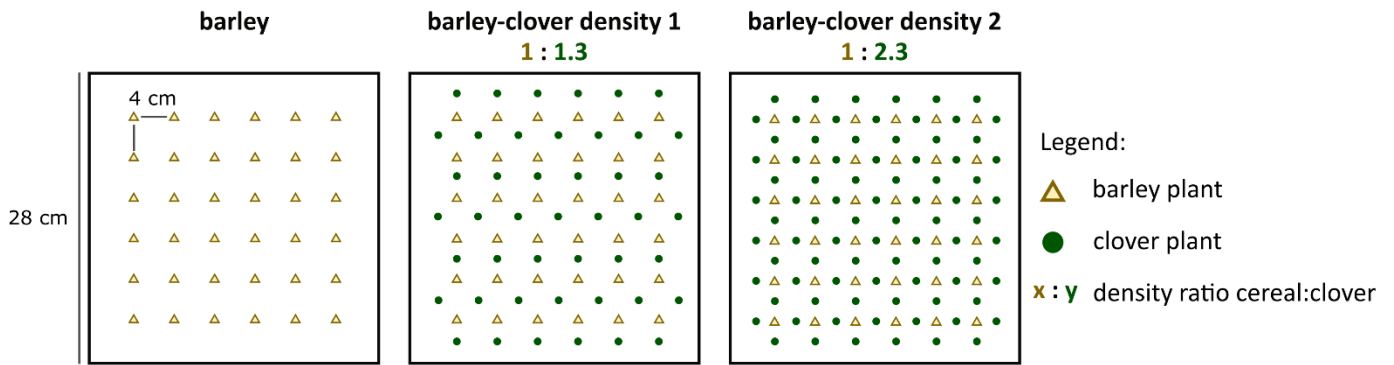


Figure 3.1. Experimental treatments sown for the arena experiment. barley (b) with 36 barley plants; barley-clover density 1 (bc1) with 36 barley plants + 45 clover plants; barley-clover density 2 (bc2) with 36 barley plants + 84 clover plants.

The *Rhopalosiphum padi* clone RplA (collected in 2012 in the Yonne department, France) was used in the experiment. Viruliferous RplA were reared on barley cv. Etincel plants infected by the isolate 4 of BYDV-PAV (BYDV-PAV4; (Chain *et al.*, 2007)) in a small plexiglass cage maintained in a growth chamber (day/night: 16 h/8 h, 25°C/20°C; RH: 40%).

2.2. Arena experiment

Square pots (b, bc1 and bc2, Figure 3.1) with clovers and/or barley plantlets were used to run arena experiments. Six days after sowing, a third instar nymph from a viruliferous RplA population was deposited at the base of the plant located at the row 3 and line 3 of the arena using a fine brush (deposit tiller). The morph (wingless or alate) of the third instar nymph, called the founder aphid, was not known at the time of deposit. The arena was covered by an insect-proof net. This experimental set-up was maintained in a growth chamber for 13 days. At the end of this period, the number of aphids per barley plant was counted. Barley plants maintained in the square pots were sprayed with insecticide (Pirimor® 0.1% v/v, Syngenta®, Basel, Switzerland). They were maintained in an insect-proof greenhouse for 3 weeks, the time at the end of which viral accumulation in the plant is maximal if the plant has been inoculated by aphids. At the end of this period, the sanitary status (presence of BYDV-PAV) of each barley plant was evaluated by an Enzyme-Linked Immuno-Sorbent Assay (ELISA).

24 replicates per treatment over 4 sessions were planned (i.e. 6 replicates per treatment per session). Due to technical reasons (i.e. arenas lost because death of the founder aphid; very poor clover emergence), 65 arenas in total were finally run.

2.3. Serological detection of BYDV-PAV in plants

Each barley plant (all leaves) was individually sampled and grounded with a Pöllhane press (MEKU®, Wennigsen, Germany). The presence of BYDV-PAV in barley plants was tested using a double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977). Wells of a microtiter plate (NUNC, Maxisorp, Thermo Fisher Scientific, Waltham, MA, USA) were incubated at 37°C for 3 h with a polyclonal anti-BYDV-PAV antibody (PAV52, H. Lapierre, INRAE) previously diluted (1/1500 (v/v)) in a carbonate buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, pH = 9.6). Between each step of the DAS-ELISA procedure, plates were washed 3 times with PBST buffer (137 mM NaCl, 8 mM Na₂HPO₄, 12H₂O, 2.7 mM KCl, 1.5 mM KH₂PO₄, 0.05% (v/v) Tween 20, 2% (w/v)). Then, 100 µL of plant sap was added into coated wells and incubated overnight at 4°C. Alkaline phosphatase conjugated-PAV52 antibody (100 µL) diluted (1/1000 (v/v)) in conjugated buffer (PBST buffer supplemented with 2% (w/v) polyvinylpyrrolidone 40T and 2% (w/v) ovalbumin) was deposited in wells

and incubated for 3 hours at 37°C. Wells were filled with 100 µL of diethanolamine (1N, pH = 9.8) containing p-nitrophenylphosphate (1 mg/mL). After incubation at room temperature in the dark for 1 h, optical density at 405 nm (OD405) was recorded for each well using a spectrophotometer (Multiskan™ FC; Thermo Scientific™, Waltham, MA, USA). A positive detection of BYDV-PAV in a tested sample was considered when the OD405 value was more than twice the OD405 value obtained for healthy control samples, with a minimum value of OD405 = 0.1.

2.4. Statistical analyses

To investigate aphid colonisation and virus spread in the arena, several parameters linked to the infestation and infection of tillers were collected and analysed with different statistical tests.

2.4.1. Population scale analysis

The effect of the founder aphid morph and the clover treatment on different variables was analysed at the population/arena scale (i.e. 1 data per arena). The variables were the distance founder tiller–deposit tiller, aphids per founder tiller, aphids per arena, proportion of infested tillers, proportion of infected tillers, proportion of infected non-infested tillers, proportion of infected infested tillers. Generalized Linear Mixed Effects Models (GLMM) were used with a session random effect on the intercept to account for potential heterogeneity in aphid population growth and virus transmission between experiments. The model included the fixed effect of the morph (2-level factor: wingless and alate morphs) in interaction with the fixed effect of the treatment (3-level factor: b, bc1 and bc2).

Variable tested ~ morph + treatment + morph × treatment + (1|session)

A gaussian link was used for distance founder tiller–deposit tiller after a square root transformation, and a binomial family link was used for occurrence variables and a negative binomial link for count variables to account for overdispersion. The significance of fixed factors and their interaction was determined by a likelihood ratio test (LRT).

2.4.2. Tiller scale analysis

The effect of the clover treatment and the distance from the founder tiller on different variables was analysed at the tiller scale (i.e. 1 data per tiller). The data were analysed separately considering the two founder aphid morphs. The variables were aphids per tiller or per infested tiller, the occurrences of aphids and virus per tiller, and the occurrences of virus per infested and non-infested tiller. To analyse the spatial spreading pattern of variables around the founder tiller, the distance from the founder tiller (numerical) was used as an explanatory variable. GLMM with a binomial link were used for occurrence variables and GAMM (Generalized Additive Mixed Effects Models) with a negative binomial link for count data that displayed non-linear responses regarding to the distance variable. The model included the fixed effect of the treatment (3-level factor: b, bc1 and bc2) in interaction with the distance from the founder tiller (number of tillers from the founder tiller). The random effect of the arena nested in the session was used to account for non-independent observations due to experimental variations of aphid population growth and virus transmission.

Variable tested ~ treatment + distance + treatment × distance + (1|session/arena)

Statistical analyses were performed using R version 4.1.1 with the lme4 package for GLMM and gamm4 package for GAMM. The drop1 function was used to perform LRT.

3. Results

3.1. Morph and virus transmission

In the 65 arenas (distributed between the 3 treatments, Table 3.1), the founder nymphs became a wingless adult in 40 arenas (61.5%) and an alate adult in 25 arenas (38.5%). During the 13 days of the experiment, two generations of aphids were produced from the founder aphid. These generations were only composed of wingless individuals whatever the morph of the founder aphid. The tiller on which the founder aphid has established the first colony, i.e., the founder tiller, hosted 72.2 ± 3.7 aphids while other tillers hosted 5.4 ± 0.2 individuals (Annexe 3). According to the viruliferous status of the founder aphid, plants (at least some of them including the founder tiller) of the arenas were expected to be infected by the BYDV-PAV4 isolate. However, analyses of the sanitary status of plants showed that infected plants were present in only 46 arenas (70.8%), suggesting that nymphs were not all viruliferous or didn't have enough virus particles to reach the infection threshold. Thus, arenas without infected plants were removed from data set used to analyse viral spread.

For interactions between treatments (b, bc1 and bc2) and aphid morphs (wingless and alate), no significant statistical link was found, neither for the 65 arenas ($p = 0.565$) nor the 46 infected arenas ($p = 0.351$, Pearson's Chi-squared test based on 106 replicates). Thus, no confounding effects are expected between these factors in the following results.

Table 3.1. Wingless and alate founder morph in arenas.

Treatment	Founder aphid morph *	
	Wingless	Alate
b	13 (6)	10 (7)
bc1	12 (11)	9 (5)
bc2	15 (12)	6 (5)

* Total number of arenas (Number of arenas containing infected plants).

3.2. Morph and clover effect at population scale

Analyses carried out at the population scale (1 data per arena) showed that clover has no significant effect on parameters linked to aphid colonisation and virus spread (Table 3.2). However, there is a strong interaction between the morph and the treatment for all monitored parameters except for aphids per founder tiller and proportion of infected non-infested tillers. For the wingless morph, the presence of clover in barley arenas tends to decrease size of aphid populations and rate of virus infections, whereas for the alate morph, these variables tend to increase (Table 3.2). For all parameters except proportion of infected non-infested tillers, morph has a highly significant effect. Thus, for arenas started with a founder aphid with a wingless morph, the size of aphid populations is 2.6 times higher (Table 3.2, Figure 3.2a), the number of aphids per founder tiller is 1.7 times higher (Table 3.2, not illustrated), and the proportion of infested tillers is 2.0 times higher (Table 3.2, Figure 3.2b) compared to arenas started with an alate founder morph.

To describe the spread of BYDV-PAV in the arena, the sanitary status of plants must be analysed together with the presence/absence of aphids on each of the 36 plants of the arena. Infection rate in the whole arena (proportion of infected tillers) and infection rate associated to infested plants (proportion of infected infested tillers, i.e., hosting aphids) were significantly lower for arenas with an alate founder morph (Table 3.2, Figure 3.2c). Proportion of infected non-infested tillers is not significantly different between the two aphid morphs (Table 3.2, Figure 3.2c). Concerning the distance between the founder tiller (tiller on which the founder aphid has established the first colony) and the

deposit tiller (tiller on which the founder aphid was deposited at the beginning of the experiment, i.e. the tiller located at the row 3 and line 3) (Annexe 3), which can reflect the early mobility of the founder aphid before it settled on a tiller, the morph has a significant effect. For arenas started with an alate morph, this distance is twice the distance observed for arenas with a wingless founder morph (Table 3.2, Figure 3.2d). Moreover, there is a slightly significant interaction between the morph and the treatment, probably explained by a distance founder tiller-deposit tiller halved for bc2 in case of alate founder morph (Table 3.2).

Table 3.2. Aphid population and virus spread at the population scale.

Variable tested	Wingless *				Alate *				p-value **			R ² ***	
	All arenas	b	bc1	bc2	All arenas	b	bc1	bc2	M	T	M × T	R ² m	R ² c
Distance founder tiller–deposit tiller	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	2.3 ± 0.2	2.8 ± 0.3	2.5 ± 0.4	1.1 ± 0.4	0.001	0.11	p < 0.001	0.19	0.19
Aphid per founder tiller	85.4 ± 3.9	93.4 ± 6.2	79.7 ± 8.3	83.0 ± 6.0	51.1 ± 4.9	42.7 ± 8.0	61.4 ± 8.8	49.5 ± 6.8	p < 0.001	0.92	0.22	0.29	0.29
Aphid per arena	342.2 ± 21.6	412.9 ± 39.1	316.3 ± 32.2	301.7 ± 34.6	132.7 ± 13.5	112.4 ± 18.4	137.9 ± 23.4	158.7 ± 2.4	p < 0.001	0.44	0.009	0.53	0.63
Infested tillers (%)	72.4 ± 2.7	80.5 ± 3.0	70.8 ± 5.7	66.7 ± 4.3	35.2 ± 3.9	28.6 ± 4.3	36.1 ± 8.7	44.9 ± 6.3	p < 0.001	0.41	p < 0.001	0.66	0.85
Infected tillers (%)	43.1 ± 5.8	50.5 ± 13.2	37.1 ± 7.7	44.9 ± 10.5	29.9 ± 3.9	24.6 ± 5.7	36.1 ± 7.5	31.1 ± 8.2	p < 0.001	0.57	0.01	0.38	0.62
Infected non-infested tiller (%)	7.2 ± 1.5	7.9 ± 2.8	7.3 ± 2.5	6.7 ± 2.5	11.6 ± 1.3	11.1 ± 2.5	13.9 ± 9.9	9.4 ± 3.2	0.11	0.39	0.78	0.06	0.33
Infected infested tiller (%)	35.9 ± 4.9	42.6 ± 11.6	29.8 ± 6.1	38.2 ± 8.9	18.5 ± 3.3	13.5 ± 3.2	22.2 ± 6.9	21.7 ± 7.8	p < 0.001	0.28	p < 0.001	0.56	0.66

b: barley; bc1: barley-clover density 1; bc2: barley-clover density 2; M: morph of the founder aphid (wingless, alate); T: treatment (b, bc1, bc2); M × T: interaction between morph and treatment.

* Mean ± SEM of all arenas combined and per treatment according to the morph of the founder aphid. ** p-values from GLMM models (p-values < 0.05 are written in bold). *** R²m: marginal R-squared value considering only the variance explained by the fixed effects; R²c: conditional R-squared value considering the variance explained by both fixed and random effects.

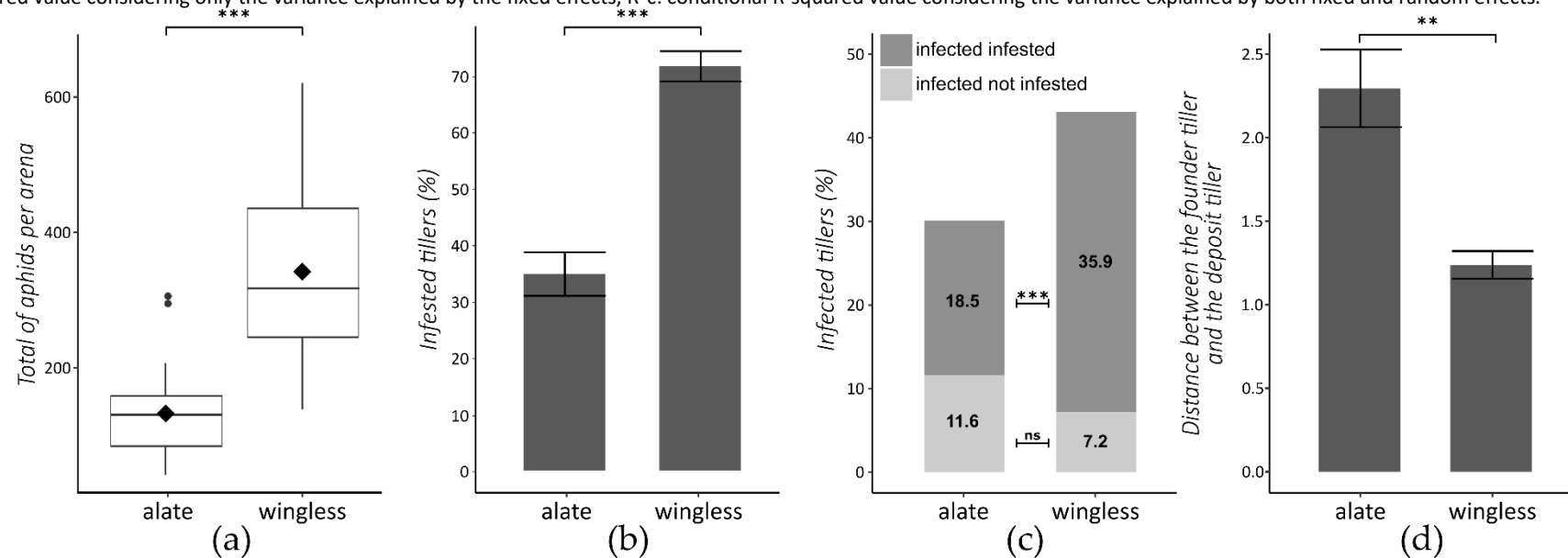


Figure 3.2. Aphid population and virus spread at the population scale, according the founder aphid morph. (a) Total number of aphids per arena (the black diamond represents the mean). **(b)** Percentage of tillers infested (Mean ± SEM); **(c)** Percentage of tillers infected; **(d)** Distance between the founder tiller and the deposit tiller.

** : p < 0.01, *** : p < 0.001, ns: not significant.

3.3. Clover and distance effect at tiller scale

To complete the description of data associated to aphid density and virus occurrence in arenas, spatial parameters have been used to evaluate the impact of clover on the overall movement of the founder aphid and its progeny, and on the viral spread. Analyses were carried out at the tiller scale (36 data per arena).

Table 3.3. Aphid colonisation and virus spread at the tiller scale.

Variable tested		Fixed factors	Wingless		Alate	
			p-value *	R ² **	p-value *	R ² **
Aphids	occurrence per tiller	Distance	p < 0.001		p < 0.001	
		Treatment	0.03	R ² m: 0.07	0.33	0.16
		D × T	0.96	R ² c: 0.18	p < 0.001	
	per infested tiller	Distance	p < 0.001		p < 0.001	
		Treatment	0.04	0.57	0.15	0.51
		D × T	0.21		0.26	
	per tiller	Distance	p < 0.001		p < 0.001	
		Treatment	0.004	0.55	0.42	0.42
		D × T	0.10		0.02	
Virus	occurrence per tiller	Distance	p < 0.001		p < 0.001	
		Treatment	0.81	R ² m: 0.03	0.79	R ² m: 0.06
		D × T	0.57	R ² c: 0.46	0.10	R ² c: 0.19
	occurrence per non-infested tiller	Distance	0.07		p < 0.001	
		Treatment	0.57	0.008	0.61	0.008
		D × T	0.81		0.90	
	occurrence per infested tiller	Distance	0.004		p < 0.001	
		Treatment	0.76	0.02	0.76	0.13
		D × T	0.40		0.07	

D: Distance to the founder tiller; T: treatment (b, bc1, bc2); D × T: interaction between distance and treatment.

* p-values from GLMM and GAMM models (p-values < 0.05 are written in bold). ** GLMM analysis: R²m: marginal R-squared value; R²c: conditional R-squared value. GAMM analysis: R²: adjusted R-squared value.

3.3.1. Wingless founder morph

Concerning arenas started with a founder aphid with a wingless morph, the spatial distribution of aphids and of infected barley plants present a 'one focus' pattern, concentrated around the founder tiller (Annexe 3). Indeed, the distance to the founder tiller has a high significant effect for all the studied parameters, except the virus occurrence per non-infested tiller (Table 3.3). With the distance to the founder tiller increased, the colonisation of aphids (aphid occurrence per tiller, aphids per infested tiller, and aphids per tiller) and the spread of the virus (virus occurrence per tiller and virus occurrence per infested tiller) decreased.

The presence of clover in the arena reduces the size of aphid populations. Indeed, the treatment had a significant effect on aphids per infested tiller (Table 3.3, Figure 3.3a), aphids per tiller (Table 3.3, Figure 3.3b), and aphid occurrence per tiller (Table 3.3, Figure 3.3c). When the clover density increases, the effect of clover is amplified for aphid occurrence per tiller (6% reduction for bc1 and 18% for bc2, compared to barley alone) and for aphids per tiller (21% reduction for bc1 and 27% for bc2). Concerning aphids per infested tiller, the effect of clover is similar for the two clovers densities (16% reduction for bc1 and 12% for bc2).

Although clover reduces the size of aphid populations in the arena, there is no effect of clover detected on the virus spread. Indeed, the treatment had no significant effect (p -values > 0.57) on virus-linked parameters (Table 3.3, Figure 3.3d). Finally, the clover has no spatial effect on aphids and virus spread. It did not modify the 'one focus' pattern. Indeed, the interaction between distance and treatment was not significant for all the variables studied (Table 3.3).

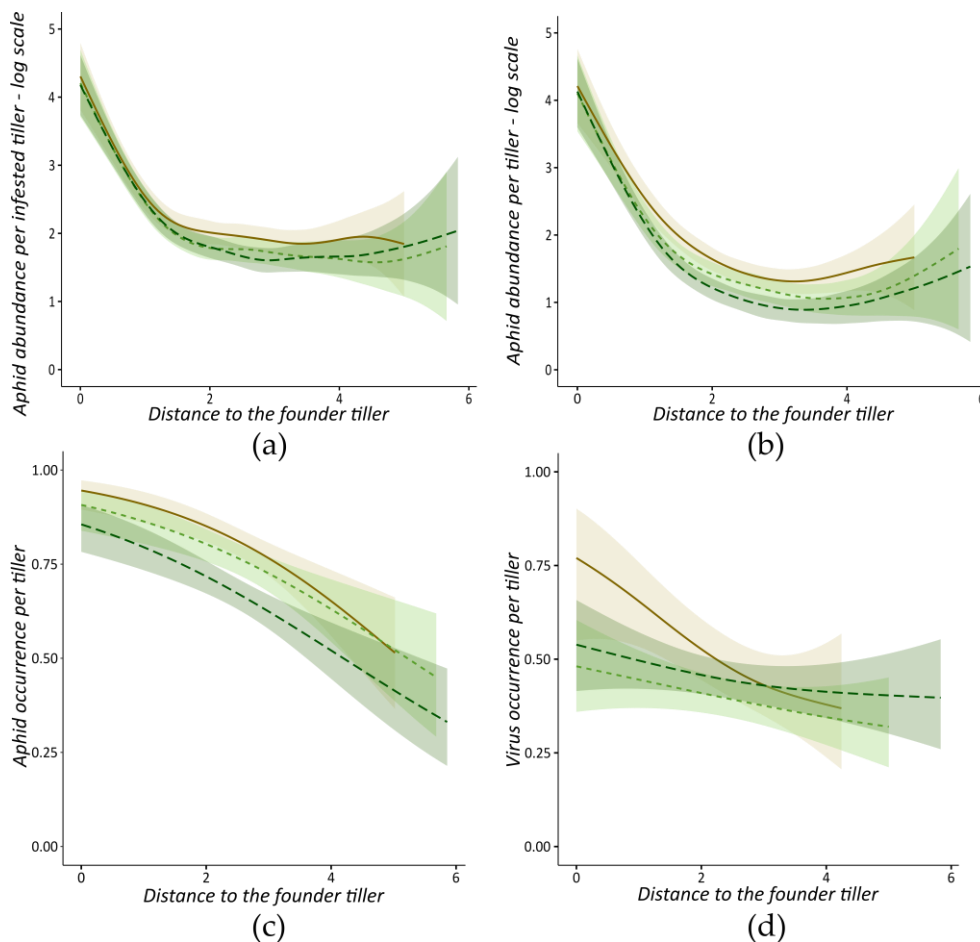


Figure 3.3. Aphid colonisation and virus spread at the tiller scale, according to the distance from the founder tiller. Arenas started with a wingless founder morph. (a) Log(Number+1) of aphids per infested tiller; (b) Log(Number+1) of aphids per tiller; (c) aphid occurrence per tiller; (d) virus occurrence per tiller. Each curve corresponds to one treatment: — : b, - - - : bc1, · · · : bc2. Areas are the 0.95 margin error for each curve.

3.3.2. Alate founder morph

Concerning arenas started with a founder aphid with an alate morph, the distance to the founder tiller had a high significant effect for all monitored parameters. As the distance to the founder tiller increased, colonisation of arena by aphids and the proportion of infected plants decreased (Table 3.3). In contrast with observations made for arenas associated to wingless founder morph, the presence of clover has no effect on aphid population size and on the spread of virus in arenas started with an alate founder morph (Table 3.3).

The clover has a negative effect on the spatial distribution of aphids. Indeed, the interaction between the distance and the treatment had a significant effect on aphids per tiller (Table 3.3, Figure 3.4b) and on aphid occurrence per tiller (Table 3.3, Figure 3.4c). According to the ‘one focus’ shape described above, for arenas bc1 and bc2, aphid occurrence and aphids per tiller decreases when distance to the founder tiller increases, until these parameters are almost null for the most distant tillers (Figure 3.4b and 3.4c). However, for b arenas, these two parameters decrease up to a distance of 4 tillers away from the founder tiller and then increase until it reaches an aphid occurrence of 0.8 and 3.7 aphids per tiller for the most distant tillers (Figure 3.4b and 3.4c). This horseshoe-shaped curve suggested the development of two distant aphid colonies, i.e. a ‘two foci’ distribution (Annexe 4), probably resulting from a “long distance” movement of the alate founder morph in the arena at an early step of the experiment.

For virus-linked parameters, the interaction between distance and treatment was not significant (Table 3.3). The clover, therefore, did not modify the shape of virus spread pattern (Figure 3.4d).

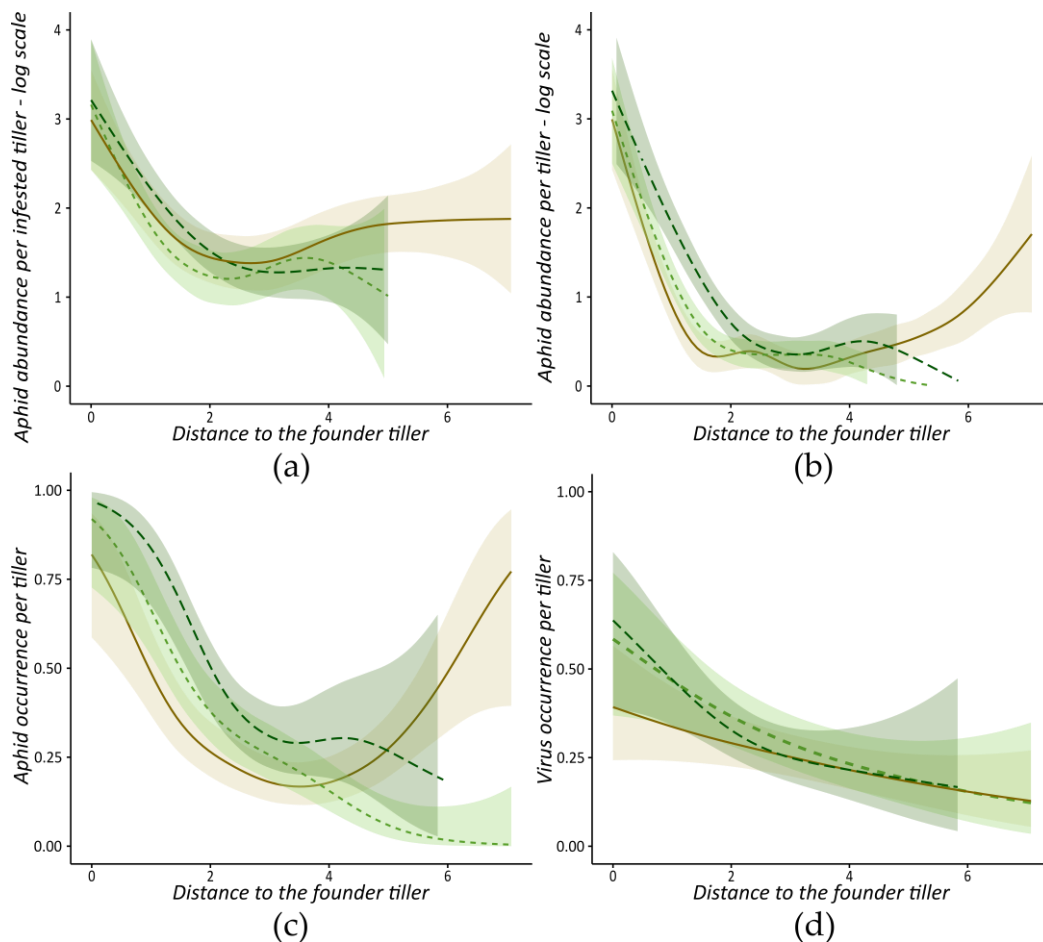


Figure 3.4. Aphid colonisation and virus spread at the tiller scale, according to the distance from the founder tiller. Arenas started with an alate founder morph. (a) Log(Number+1) of aphids per infested tiller; (b) Log(Number+1) of aphids per tiller; (c) aphid occurrence per tiller; (d) virus occurrence per tiller. Each curve corresponds to one treatment: —: b, - - - : bc1, ··· : bc2. Areas are the 0.95 margin error for each curve

4. Discussion

The introduction and spread of insect-borne viral disease in a field is mainly based on biological parameters of virus–plant interactions. However, the vector, through its interactions with the host plant and with the virus, plays also a major role in this process. Indeed, it will determine both efficiency and dynamics of plant-to-plant virus transmission. In the absence of a chemical treatment directly against viruses and in an agronomic context that lacks genetic solutions with resistant crop varieties to efficiently control the disease in fields, chemical solutions targeting vectors of plant viruses have often been preferred. In order to develop agroecological methods to control viral diseases, the impact of a non-host plant (i.e. clover plant) on the biological and spatial parameters of the epidemiology of barley yellow dwarf disease was, therefore, tested.

Our experiment with barley plantlets and conducted with or without the presence of clover did not reveal an overall general benefit of clover on the dynamics of aphid populations nor on the disease spread. However, the analysis, by separating the two founder morphs, shows differentiated effects of the non-host clover on parameters linked to the epidemiology of the aphid-transmitted yellow dwarf disease.

The initial step of field colonisation by aphids is the introduction of alate individuals from wild or cultivated reservoirs located outside the field (Henry and Dedryver, 1989; Henry and Dedryver, 1991). When these individuals reach a field grown with their host plant, alate aphid land, probe, and fly between several plants before settling down in a more sedentary manner (Moericke, 1955). We observed, through our measurements in the arenas with barley alone, distribution patterns that could well be explained by these “trivial flights”. Indeed, several founder sites of aphids were observed within the arenas initiated with an alate founder morph. This initial mobility of founder alate individuals is altered in our experimental set-up by the presence of clover between barley plants, which limits the number of aphid founder sites to one. Another parameter shows that alate mobility is altered in the presence of clover in our set-up. Indeed, the distance founder tiller-deposit tiller is reduced by 2.5 for bc2 arenas compared to barley alone. The first day of the experiment, the clover was at the cotyledon stage, and it developed to reach the 1-leaf stage at the time the founder aphid reached the adult stage (not illustrated). Thus, when the alate individuals start plant-to-plant movements in the arena, the clover is in average 5 cm high. It is surprising that the spatial dynamics of the aphids can be altered by the presence of these small non-host plants, since alate can fly without being disturbed by the physical barrier that the clover might represent at this step of the experiment. It cannot be ruled out that, under our experimental conditions, the movements of the alate aphid from multiple founder sites occurs through ground movements by walking on the soil from plant-to-plant. However, the clover plantlets would also participate to the modification of the environment at olfactory and/or visual levels, disturbing spatial movement of alate founder *R. padi*. Thus, based on data collected from arena design, the process of introduction of alate aphids in barley fields, which is one of the most important steps for the incidence of YDD at field scale (i.e. intensity of primary inoculations), could be spatially disturbed by the presence of a non-host plant such as clover, reducing the number of founder sites per migrant alate aphid.

From these founder sites initiated by alate individuals in the field, several generations of wingless aphids will develop by parthenogenesis, increasing the size of the founder site by colonising neighboring plants (Hodgson, 1991). Population density is known to be an important parameter in the colonization of space by aphids. The presence of clover in our work showed a significant decrease in several descriptive parameters of aphid populations generated from a wingless founder morph.

Indeed, the number of aphids per infested tiller and the percentage of plants infested were reduced in the presence of clover. This result is consistent with other studies testing the effect of plant diversification on aphid populations (Döring, 2014). The non-host plant can disturb aphid behaviour by different mechanisms. In the presence of clover, aphids may spend more time on the non-host plant at the cost of the host plant, leading to a decrease of the total size of the produced population. When the founder aphid has an alate morph, we do not observe a reduction of the following aphid populations in the presence of clover. However, it is important to note that the total number of aphids produced in an arena by the alate founder morph was 2.6 times lower than the population produced by the wingless founder morph. It has been reported that the two aphid morphs present different life history traits, especially a longer nymphal development and a lower offspring production for alate aphids (Braendle *et al.*, 2006). Khan and Port (2008) reported a fecundity of alate *R. padi* reduced by half compared to wingless adult. The effect of clover on *R. padi* abundance may be visible only with high-density populations, as shown in a previous study on genetic plant diversification (Power, 1991), which was not the case under our experimental conditions with alate aphids. Then, the spatial effect of the clover observed in arenas initiated by an alate founder morph was not observed in the case of the wingless morph. Since in both morphs the following generations in arenas are wingless aphids, the spatial effect of the clover acts only on the founder individual. This absence of spatial effect of clover can be explained by the low mobility of the wingless adult, supported by the distance founder tiller-deposit tiller measurement, which is on average only 1 tiller. Thus, the observations made in this study show that the dynamics of extension of the initial infestation sites, ensured by the dispersal from plant-to-plant of wingless individuals, is partly lowered by the presence of clover.

Despite the effect of clover on the abundance of aphids (founder wingless morph) or on the distribution of aphids (founder alate morph), we did not detect an effect of the clover on the BYDV incidence. Vector abundance is obviously an important parameter in the epidemiology of viral disease. However, as described in numerous studies, vector behaviour (i.e. feeding behaviour or dispersal behaviour) should be considered as one of the key parameters for virus spread (Rochow, 1974; Harrewijn, 1989). The efficiency of plant-to-plant virus transfer depends on both the efficiency of acquisition of viral particles from an infected plant and the inoculation of virus to a healthy host. These two steps rely on characteristics of feeding behaviour of the insect vector. This feeding behaviour could be modified in an intercropping system compared to a monoculture one. For example, it has been shown that leafhoppers, which are plant sucking insects, spent more time on their host plant in a genetically diversified crop system, leading to an increase in the spiroplasma transmission rate (Power, 1988). Thus, further studies are needed to understand whether the non-homogeneous environment constituted by the association of barley and clover may modify *R. padi* feeding behaviour. Concerning the dispersal behaviour of insects in a diversified habitat, the non-host plant could increase insect mobility, increasing the number of plants visited and infected. In our experiment, the virus occurrence per non-infested tiller allowed us to test this mobility at the population scale. This parameter remains unchanged in the presence of clover, whatever the morph of the founder aphid.

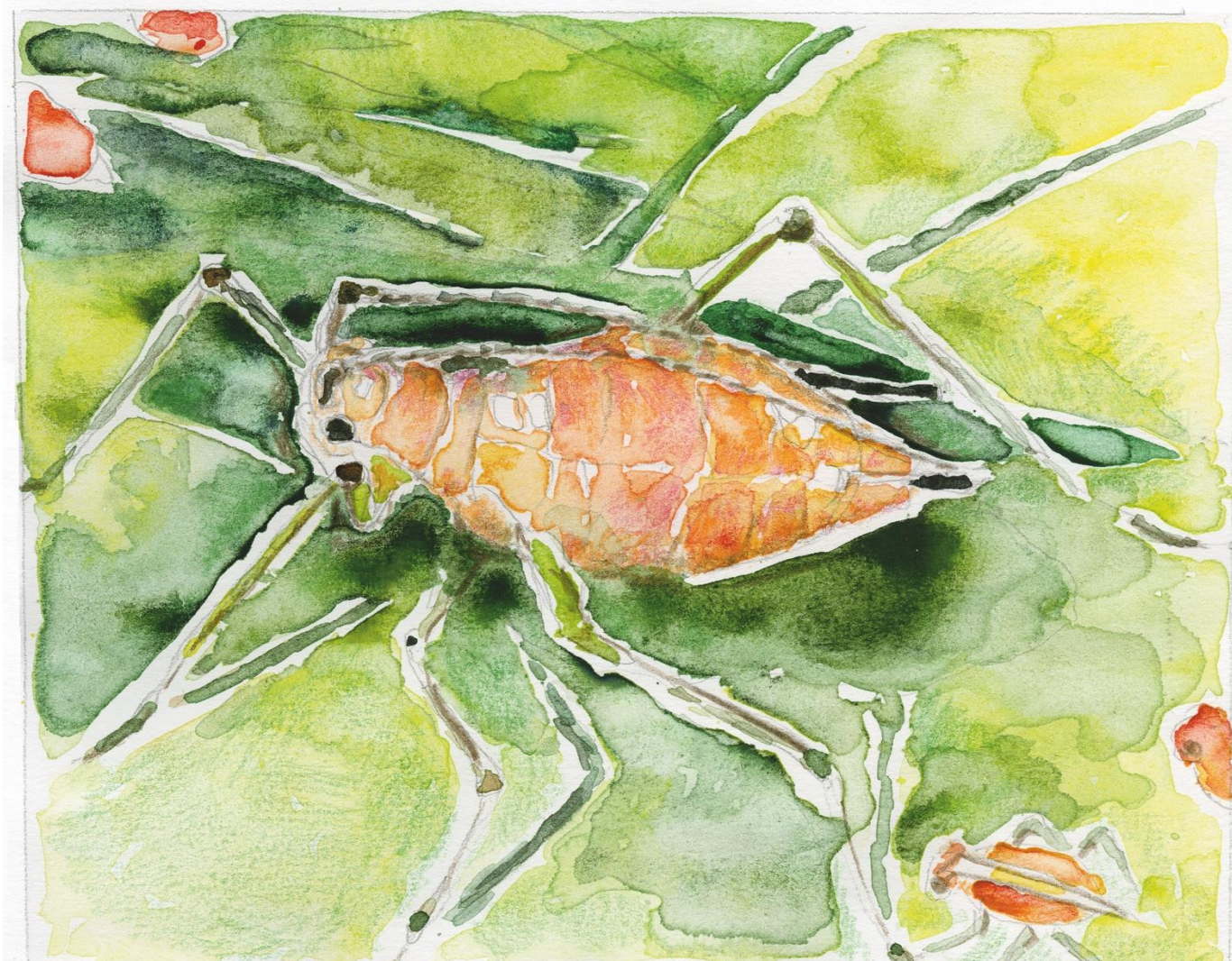
In a plant-insect-virus pathosystem, the virus can also influence the insect behaviour. Concerning *R. padi* and BYDV, several studies showed that virus-free *R. padi* were more attracted by infected plants (Medina-Ortega *et al.*, 2009; Ingwell *et al.*, 2012; Fingu-Mabola *et al.*, 2020), particularly by the VOCs emitted by these plants (Jiménez-Martínez *et al.*, 2004), while viruliferous *R. padi* preferred healthy hosts (Ingwell *et al.*, 2012; Fingu-Mabola *et al.*, 2020). This is an example of the “vector manipulation” hypothesis, commonly found for insect-borne viruses (Mauck *et al.*, 2016). This vector manipulation can explain why *R. padi* abundance is decreased in our experiments in the

presence of clover, but not the BYDV incidence. Finally, it cannot be excluded that clover has an impact on BYDV but that the effect was not observed under our experimental design.

The arena-based experiment carried out using barley/clover associations gives indications regarding the presence of clover and the ability of *R. padi* to colonise cultivated barley fields. Growing these two plant species together would reduce (i) the number of founder sites initiated by alates for the development of offspring, (ii) the number of infested barley plants by wingless aphids, and (iii) the size of aphid colonies. Thus, the presence of clover plants in barley growing areas would participate to lower the risks associated to the presence of BYDV vectors through a bottom-up regulation of aphid populations within the field. To better characterize if and how barley/clover intercropping would reduce BYDV prevalence, further laboratory experiments and field trials are needed.

Acknowledgments: We thank Marlène Souquet, Luâna Korn, and Melissandre Barbet for their technical assistance and support.

Funding: This work was supported by the European Union's Horizon 2020 Research and Innovation programme, under Grant Agreement no. 773554 (EcoStack).



Aquarelle, *Sitobion avenae*
Christine Grauby

CHAPITRE 4

EFFET DE LA DIVERSIFICATION VEGETALE INTRA-PARCELLAIRE
SUR LES PUCERONS DES CEREALES, LA JAUNISSE NANISANTE
DE L'ORGE ET LES ENNEMIS NATURELS DES PUCERONS, AU
DEBUT DU CYCLE D'UNE CEREALE D'HIVER

Chapitre 4. Effet de la diversification végétale intra-parcellaire sur les pucerons des céréales, la jaunisse nanisante de l'orge et les ennemis naturels des pucerons, au début du cycle d'une céréale d'hiver

Part 1. Effect of intra-field plant diversification on cereal aphids, vectors of the Yellow dwarf disease, at the beginning of a winter cereal growing cycle

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Keywords: Yellow dwarf disease; cereal aphids; intercropping; cultivar mixture; barley

Abstract: Yellow dwarf disease (YDD), one of the most important viral diseases in cereal crops worldwide, induces dwarfing, leaf discoloration and yield losses up to 80%. The disease is caused by a complex of ten virus species (e.g. the PAV species of Barley yellow dwarf virus (BYDV-PAV) is the most prevalent species in France) transmitted in a persistent non-propagative manner by at least twenty-five aphid species. Control methods used by farmers against YDD are limited, whether in conventional farming where the availability of plant protection products and their effectiveness is decreasing, or in organic farming where the effectiveness of late sowing (i.e. the main control method used) tends to decrease with global warming. Intra-field plant diversification is a promising strategy to reduce insect pest populations and can be implemented through intra- or inter-specific diversifications. Studies have shown promising effects of these two types of diversification on cereal aphids. However, most of these studies, carried out in temperate climate regions, were conducted in spring when i) companion plants are well developed and ii) aphid's natural enemies can be abundant. To our knowledge, no field study has been performed on the potential of plant diversification to regulate cereal aphid populations at the beginning of the winter crop cycle, when cereals are highly susceptible to aphid-mediated inoculation of BYDV-PAV. The objective of this study was therefore to test, in on-farm field trial, the potential of intra- or inter-specific plant diversifications i) to disrupt alate aphid introductions into fields and ii) to decrease the spread of aphids on cereals and of BYDV incidence. Results showed that plant diversification do not modify monitored parameters of the epidemiology of YDD. However, it is important to note that clover was, from November to December, poorly developed in the field trials, which may have caused limited disturbance to the aphid vectors during the monitored period.

1. Introduction

Yellow dwarf disease (YDD), caused by a complex of ten virus species, is one of the most important viral diseases in cereal crops worldwide, causing yield losses up to 80% (Nancarrow *et al.*, 2021). In temperate climate regions, the most important cereal aphid species transferring the virus are *Rhopalosiphum padi* (Linnaeus, 1758), *Metopolophium dirhodum* (Walker, 1849), *Sitobion avenae* (Fabricius, 1775) and *Schizaphis graminum* (Rondani, 1852) (Van den Eynde *et al.*, 2020). All of them

are able to transmit BYDV-PAV, the most prevalent and damaging virus species reported in French cereal fields (Lister and Ranieri, 1995). In early autumn, alate cereal aphids leave their summer hosts (maize, volunteers, pasture grasses) and colonise newly sown winter cereals. Some viruliferous individuals can introduce BYDV within the crop, this step of the epidemiological cycle is called the primary infection. Once settled on their host plant, alate aphids can also produce offspring, which are mainly apterous. If offsprings acquire the virus by feeding on an infected plant, they can disperse it through short plant-to-plant dispersal, this is the secondary infection step (Lapierre and Signoret, 2004). Global warming, with increasingly mild autumns and winters, will have consequences on several parameters of aphid development and BYDV epidemiological cycle (Habekuss *et al.*, 2009). In mild autumns, alate aphid flights can occur over a longer period and with higher flight intensity (Jones, 2016; Bell *et al.*, 2015), increasing the risk of primary infection. In mild conditions, individuals present in the field can remain active throughout the winter, increasing the risk of secondary infection.

In conventional farming, there are major challenges for YDD control with the reduction of plant protection products available, and in particular the ban on neonicotinoids in 2018, but also the emergence of insecticide-resistant aphids (Walsh *et al.*, 2020; Zhang *et al.*, 2020). In organic farming, late sowing of winter cereals is widely used to avoid the aphid flight period, but the effectiveness of this technique decreases with mild autumns, becoming increasingly frequent. Thus, in a context of global warming, reduction of pesticide availability and use, and the development of organic farming, it is necessary to find new strategies to reduce cereal aphid populations in winter and decrease BYDV incidence.

Intra-field plant diversification is a promising strategy to reduce insect pest populations (Hooks and Johnson, 2003; Letourneau *et al.*, 2011; Dassou and Tixier, 2016). It can be implemented through intra-specific or inter-specific diversification. Intra-specific plant diversification is the use of two or more varieties of the same species, grown together. Laboratory, greenhouse and field studies have shown that intra-specific plant diversification can reduce cereal aphid populations development through: i) reducing mother aphid size and offspring production (Shoffner and Tooker, 2013; Grettenberger and Tooker, 2016), and ii) modifying aphid acceptance and their settling on plants (Pettersson *et al.*, 1999; Ninkovic *et al.*, 2002; Dahlin *et al.*, 2018). Inter-specific plant diversification, also called intercropping, is “the cultivation of at least two plant species simultaneously in the same field” (Andrews and Kassam, 1976). Recent laboratory studies found i) a 1/3 reduction of *Sitobion avenae* population when wheat was intercropped with white clover (Mansion-Vaquié *et al.*, 2020), and ii) a ¼ decrease of *Rhopalosiphum padi* population on barley in presence of crimson clover (Grauby *et al.*, 2022). Bottom-up mechanisms were suggested in this aphid regulation, the clover disrupting aphids by various disturbances (visual, olfactory, physical). In the field, several studies also reported a decrease in aphid populations in an intercropping situation (Helenius, 1989; Hesler *et al.*, 2000; Wang *et al.*, 2009; Lopes *et al.*, 2015; Xu *et al.*, 2018; Alarcón-Segura *et al.*, 2022). However, in these field studies, aphids were monitored during the spring/summer period, when crops are well developed and aphid natural enemies potentially abundant, making difficult to disentangle the underlying mechanisms of aphid regulation. This can include direct effects of the non-host plant on aphid via diverse disturbances, i.e., bottom-up regulation, or indirect effects of the non-host plant on the aphid via natural enemies i.e., top-down regulation.

In the case of YDD, the main yield losses on winter cereal are due to autumnal aphid infestations, early stages of the cereal (from emergence to tillering) being particularly susceptible to BYDV (Smith and Sward, 1982). To our knowledge, no field study has been performed on the potential of plant

diversification to regulate cereal aphid populations at the beginning of the winter crop cycle. The objective of this study was therefore to test the potential of plant diversification to disrupt alate aphid introductions into the field and to decrease the spread of aphids and BYDV within the field. Each type of plant diversification (intra- or inter-specific) was tested separately as well as in combination in order to observe if a synergistic effect exist. The following hypotheses were tested:

- 1) Each type of diversification itself reduces the introduction of alate aphids into the field, aphid populations on the cereal, and BYDV spread.
- 2) The combination of the two types of diversification allows greater effects on aphids and on virus than the addition of their individual effects.

2. Materials and methods

2.1. Crop variety selection and undersown cover crop

Prior to the implementation of the field experiment, a lab screening following the protocol described in Ninkovic *et al.* (2002), was conducted in order to determine the best possible pairwise combinations among three French candidate barley varieties to reduce aphid acceptance. Amistar and Margaux together was the best combination and allowed a 23% reduction of *Rhopalosiphum padi* acceptance (Ninkovic 2019, not published). Thus, these two winter barley (*Hordeum vulgare* L.) varieties were used, both varieties being tolerant to BYDV, and allowing an early sowing of field experiments in autumn without risking major yield losses for farmers.

The undersown clover species mixture was composed of *Trifolium alexandrinum* (*T. alexandrinum*), *Trifolium incarnatum* (*T. incarnatum*) and *Trifolium repens* (*T. repens*) species. This mixture provides a good soil cover of the sowed areas during the barley growing season.

2.2. Field experiment design

Field experiments were implemented on commercial farms under organic agriculture in the Rhône-Alpes region (South-East of France) over two consecutive winter barley growing seasons (2019-2020 and 2020-2021). Each field experiment was composed of six different treatments: three treatments with barley alone, i.e., barley variety Amistar, barley variety Margaux, and a mixture of the two varieties; and three treatments with barley associated with clover, i.e., barley variety Amistar/clover, barley variety Margaux/clover and a barley variety mixture/clover. Each treatment was seeded on a plot of 24 m x 50 m (Figure 4.1). The winter barley was sown at 150 kg/ha (355 seeds/m²), with a substitutive design for the barley mixture. The clover cover was sown at 13 kg/ha using a mixture at the rate of 5 kg/ha for *T. alexandrinum* and *T. incarnatum* and of 3 kg/ha for *T. repens*. Winter barley and clover were sown simultaneously in the respective treatments. The field experiment was set up on five organic fields in 2019 and five others in 2020. Due to bad weather conditions during autumn 2019, the field experiments were sown either on October 15th (n=2, regular sowing) or on October 30th (n=3, late sowing). In 2020, all field experiments were sown on October 15th (n=5, regular sowing). Field experiments were not subjected to any pesticides and weeds were mechanically controlled (one harrowing pass between November and March) except in the clover growing areas. Fields were fertilised once (in January/February) with organic fertilisers.

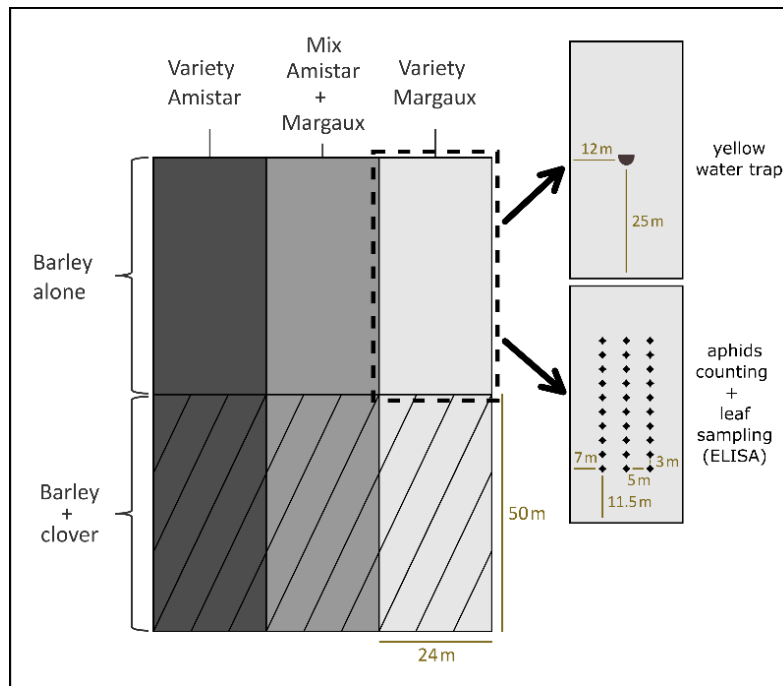


Figure 4.1. Design of the field experiment and protocols implemented in autumn-winter. Two factors are tested in six treatments: the "variety" factor (Amistar, Margaux and Mixture) and the "clover" factor (with or without clover).

2.3. Monitoring of aphids and virus

2.3.1. Monitoring of alate aphids

Alate aphids were monitored using yellow water traps, with one trap placed in the centre of each treatment (Figure 4.1). Traps contained water supplemented with few drops of odourless and colourless soap. This aphid monitoring started two weeks after sowing and lasted until mid-December. In 2019, caught insects were collected every 3-5 days due to technical reasons and field planning (9 and 7 capture events in total for the regular and the late sowing respectively). In 2020, caught insects were collected every 7 days (7 capture events in total). Aphids were identified in the laboratory at species level, and they were categorised into two groups: "non-cereal aphids" and "cereal aphids".

2.3.2. Monitoring of aphids on barley plants

Aphids within the field were monitored on 30 barley plants sampled along three parallel transects per treatment (Figure 4.1). Per transect, aphids were counted on 10 plants, with a distance of 3 m between two plants. Within each treatment plot, the three transects were spaced 5 m apart, and 7 m from the edges of the plot (Figure 4.1). Each sampled plant was carefully cut and the number of aphids on it was recorded. For each aphid individual, species, development stage (nymph or adult) and adult morph (alate or wingless) were recorded. This aphid monitoring started when the first barley leaf was emerged and was carried out every two weeks until mid-December (i.e. for regular sowing monitoring started two weeks after sowing for a total of four sampling periods, for late sowing it started three weeks after sowing for a total of two sampling periods). An additional sampling period of aphids took place at the end of February.

2.3.3. Monitoring of BYDV-PAV

In order to conduct BYDV-PAV occurrence analysis, barley leaf sampling was carried out on 30 plants per treatment (the same as aphids counting) (Figure 4.1). On each plant, the last two developed leaves were collected and packaged together in a plastic bag. The bags were stored at

-20°C, until the presence of the virus in each sample was determined by serological analysis. Two sampling periods were performed in 2019 (mid-November, end of February) and four sampling periods in 2020 (mid-November, mid-December, early April, early June).

Each barley plant (two last leaves) was individually sampled and grounded with a Pöllhane press (MEKU®, Wennigsen, Germany). The presence of BYDV-PAV in barley plants was tested using a double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977). Wells of a microtiter plate (NUNC, Maxisorp, Thermo Fisher Scientific, Waltham, MA, USA) were incubated at 37°C for 3 h with a polyclonal anti-BYDV-PAV antibody (PAV52, H. Lapierre, INRAE) previously diluted (1/1500 (v/v)) in a carbonate buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, pH = 9.6). Between each step of the DAS-ELISA procedure, plates were washed 3 times with PBST buffer (137 mM NaCl, 8 mM Na₂HPO₄, 12H₂O, 2.7 mM KCl, 1.5 mM KH₂PO₄, 0.05% (v/v) Tween 20, 2% (w/v)). Then, 100 µL of plant sap was added into coated wells and incubated overnight at 4°C. Alkaline phosphatase conjugated-PAV52 antibody (100 µL) diluted (1/1000 (v/v)) in conjugated buffer (PBST buffer supplemented with 2% (w/v) polyvinylpyrrolidone 40T and 2% (w/v) ovalbumin) was deposited in wells and incubated for 3 h at 37°C. Wells were filled with 100 µL of diethanolamine (1 N, pH = 9.8) containing p-nitrophenylphosphate (1 mg/mL). After incubation at room temperature in the dark for 1 h, optical density at 405 nm (OD₄₀₅) was recorded for each well using a spectrophotometer (Multiskan™ FC; Thermo Scientific™, Waltham, MA, USA). A positive detection of BYDV-PAV in a tested sample was considered when the OD₄₀₅ value was more than twice the OD₄₀₅ value obtained for healthy control samples, with a minimum value of OD₄₀₅ = 0.1.

2.4. Monitoring of crop development

The development of the different crops was monitored in 0.25 m² quadrats, randomly replicated three times per treatment. In each quadrat, the percentage of barley and clover soil cover was recorded by visual estimations. Then, the average height of each crop was measured (barley and the three clover species) and their growing stage was recorded. In 2019, crop development was assessed at two sampling periods (end of November and mid-December). In 2020, crop development was monitored weekly from the beginning of November to mid-December and one last sampling period was held at the end of February.

2.5. Monitoring of temperatures

In the field experiments, temperatures (maximum and minimum) were recorded every 30 minutes using a Tiny-tag data logger (Tinytag plus 2, Gemini Data Loggers, Chichester, UK). Data loggers were kept during the whole experiment period.

2.6. Statistical analyses

Based on data collected on field experiments, aphid occurrence (i.e. the probability of encountering at least one aphid per plant or per yellow trap) and aphid abundance (i.e. the number of aphids per plant or per yellow trap) were calculated for cereals aphids (all species grouped together) and individually for *Rhopalosiphum padi* and *Sitobion avenae*. For the virus, BYDV-PAV occurrence per plant was calculated. These variables were analysed using Generalized Linear Mixed Effects Models (GLMM). The factor *variety* (3-levels factor: Amistar, Margaux and Mixture), the factor *clover* (2-levels factor: with and without clover) and their interaction were used as fixed effects. A random effect of the site and a random effect of the sampling period were added to account for variations of aphid populations and virus occurrence between sites and between sampling periods.

Variable tested ~ variety + clover + variety × clover + (1|sampling period) + (1|site)

A binomial family link was used for occurrence variables and a negative binomial link for abundance to account for overdispersion. The significance of fixed factors and their interaction was determined by a likelihood ratio test (LRT). Statistical analyses were performed using R version 4.1.1 with the lme4 package for GLMM. The drop1 function was used to perform LRT.

3. Results

3.1. Aphid populations in the field experiment

3.1.1. *Alate aphid introduction into the field experiment*

During the 6-weeks period of monitoring (from early November to mid-December), 905 aphids in 2019 and 800 aphids in 2020 were caught in yellow water traps on the field experiments. These aphids were collected from 234 traps in 2019 and 210 traps in 2020, indicating that very few aphids flew in the vicinity of field experiments during the surveyed periods (0.83 ± 0.05 and 0.55 ± 0.02 aphids/water trap/day in autumns 2019 and 2020, respectively). Moreover, caught aphids were mostly non-cereal aphid species with *Myzus persicae* being the most prevalent species (Table 4.1). Cereal aphids represented only 21 % in 2019 and 19 % in 2020 of aphids caught. *Rhopalosiphum padi* (*R. padi*) was the dominant species but with still low average daily catches (0.11 ± 0.01 and 0.07 ± 0.00 aphids/water trap/day in autumns 2019 and 2020, respectively). *Sitobion avenae* (*S. avenae*) was the second most abundant cereal aphid species, although 4 times less abundant than *R. padi* and nearly absent of catches in 2020 (Table 4.1).

Table 4.1. Alate aphid species caught in yellow water traps in barley fields in the two years of monitoring.

	Species	Year *		Total *
		2019	2020	
Cereal aphid	<i>Rhopalosiphum padi</i>	113 (13%)	96 (12%)	209 (12%)
	<i>Sitobion avenae</i>	52 (6%)	3 (0%)	55 (3%)
	<i>Rhopalosiphum maidis</i>	15 (2%)	31 (4%)	46 (3%)
	<i>Metopolophium dirhodum</i>	11 (1%)	11 (1%)	22 (1%)
	<i>Sitobion fragariae</i>	1 (0%)	10 (1%)	11 (1%)
	<i>Schizaphis graminum</i>	0 (0%)	2 (0%)	2 (0%)
Non cereal aphid	<i>Myzus persicae</i>	619 (68%)	367 (46%)	986 (58%)
	<i>Hyperomyzus lactucae</i>	45 (5%)	4 (1%)	49 (3%)
	Other	49 (5%)	276 (35%)	325 (19%)
Total		905	800	1705

* Total number of aphids per species and relative proportion (in brackets) for each year of monitoring and for both years combined.

The temporal flight of the two major cereal aphid species, *R. padi* and *S. avenae*, showed different patterns (Figure 4.2). For the two monitored periods, the peak of *R. padi* catches occurred at the beginning of November, when young barley plants emerged from the soil. As the temperature decreased, *R. padi* catches dropped until mid-November. In 2019, *R. padi* catches increased several times, around November 20th, November 28th and December 18th, which can be related to rising temperatures (Figure 4.2). In 2020, a small increase in *R. padi* catches occurred in early December, correlated with a rise in temperatures. Then, the catches remained almost null until the end of the monitoring period. Concerning *S. avenae*, a high increase in catches occurred in early December 2019, with higher intensity than catches of the beginning of the monitoring period (Figure 4.2).

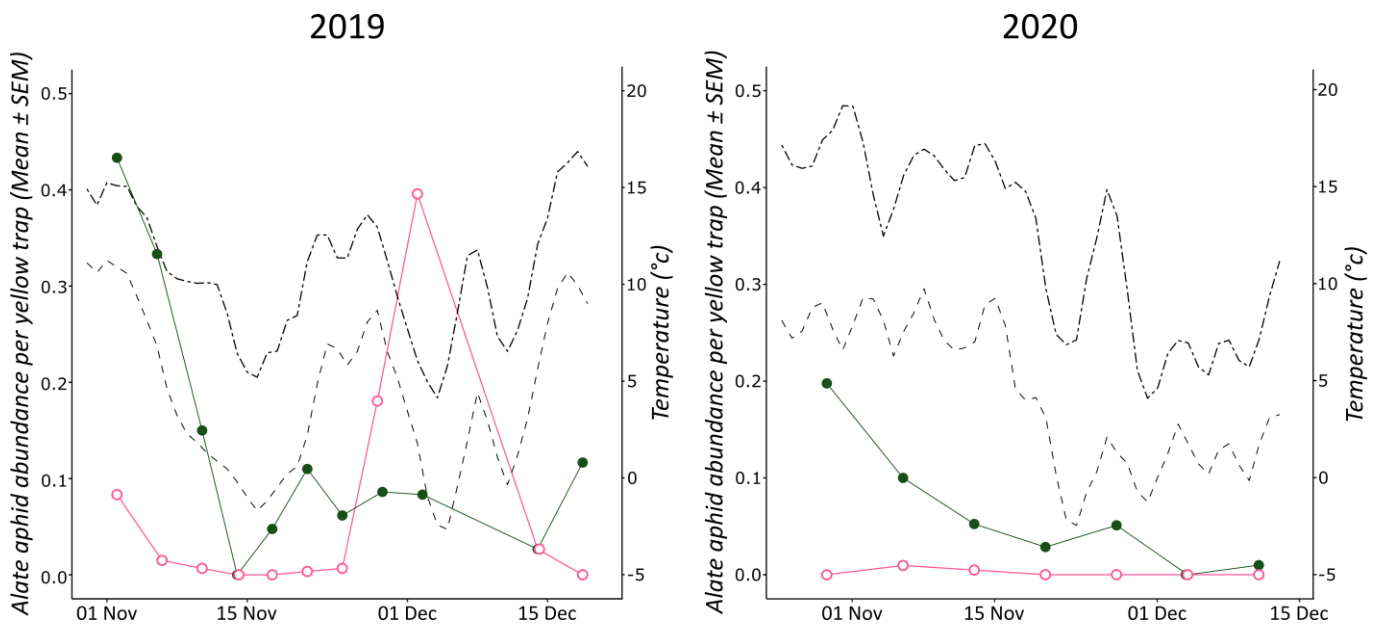


Figure 4.2. Temporal flight of alate aphids in the autumn monitoring period. Average daily catch of alate aphids per yellow water trap field trials in 2019 and 2020. *R.padi* (—●—); *S.avenae* (—○—). Mean daily temperatures (in °C) recorded on trials, averaged over 3 days (- - - - - : maximum temperature and — — : minimum temperature).

3.1.2. Aphid populations on barley plants

During the five sampling periods (from early November to February), 1817 and 2851 wingless aphids were counted on the field experiments in 2019 and 2020, respectively. In 2019, a significant inter-site difference in the size of aphid populations could be noticed between the two field experiments sown two weeks earlier on October 15th (regular sowing period) and the three other fields sown on October 30th (late sowing period) (Table 4.2). The three fields sown later showed very low aphid populations (maximum 69 aphids per site for 900 observed barley plants) suggesting that these sites were less subject to aphids' introduction and maintenance on plants in the field (Table 4.2).

Table 4.2. Total number of aphids counted on barley plants in autumn-winter per site.

Year	Sowing date	Total
2019	October 15 th	857
		856
	October 30 th	69
		23
2020	October 15 th	12
		967
		776
		522
		360
		226

The 4668 aphids observed on cereals belonged all to cereal aphid species suggesting that the non-cereal alate aphid species caught in yellow water traps did not produce offsprings in barley (Table 3). As noticed from alate cereal aphids present in yellow traps, *R. padi* was the main species in both 2019 and 2020, representing in total 50% of counts. *S. avenae* was also fairly abundant being in 2019 and 2020, the second and the third most abundant species respectively, representing in total 26% of counts. While totally absent in 2019, *Rhopalosiphum maidis* represented 28% of the counts in 2020 being the second most important cereal aphid species this year (Table 4.3).

Table 4.3. Aphid species observed on barley plants in autumn-winter in the two years of monitoring.

Species	Year *		Total *
	2019	2020	
<i>Rhopalosiphum padi</i>	1055 (58%)	1273 (45%)	2328 (50%)
<i>Sitobion avenae</i>	639 (35%)	562 (20%)	1201 (26%)
<i>Rhopalosiphum maidis</i>	0 (0%)	813 (28%)	813 (17%)
<i>Schizaphis graminum</i>	0 (0%)	87 (3%)	87 (2%)
<i>Metopolophium dirhodum</i>	14 (1%)	16 (1%)	30 (1%)
<i>Other cereal aphids sp.</i>	109 (6%)	100 (3%)	209 (4%)
Total	1817	2851	4668

* Total number of aphids per species and relative proportion (in brackets) for each year of monitoring and for both years combined. n=3420 plants observed in 2019 and n=4500 plants observed in 2020.

To complete the data set associated to aphid demography in fields, the development stage of each individual was recorded. Aphids were mainly nymphs (82%). Among adults, the wingless form was predominant compared to the alate form, being 14% and 4% of total counts, respectively (Table 4.4).

Table 4.4. Development stage of aphids on barley plants in autumn-winter in two years of monitoring.

		Year *		Total *
		2019	2020	
Adult	Alate	117 (6%)	59 (2%)	176 (4%)
	Wingless	334 (18%)	321 (11%)	655 (14%)
	Nymph	1366 (75%)	2471 (87%)	3837 (82%)

* Total number of aphids per development stage and relative proportion (in brackets) for each year of monitoring and for both years combined. n=3420 plants observed in 2019 and n=4500 plants observed in 2020.

Aphid occurrence and aphid abundance on barley plants show similar temporal patterns during the field experiment in the two years (Figure 4.3a, 4.3b). In 2019, over the first four sampling periods (from November to mid-December), on average 47% of plants were colonised by cereal aphids, with 1.2 aphids per plant. At the end-February sampling period, the populations were significantly lower with 9% of plants colonised and 0.1 aphid per plant on average. The two main species of cereal aphids showed population maximum at different periods in 2019: at the mid-December sampling period for *R. padi* and at the mid-November one for *S. avenae* (Figure 4.3a, 4.3b). In 2020, cereal aphid occurrence and abundance gradually increased from the first sampling period of November to mid-December to reach a maximum of 37% of plants colonised by aphids and 1.1 aphids per plant. At the end-February sampling period, populations were slightly lower with 30% of plants colonised by aphids and 0.6 aphids per plant, being higher populations at the end of winter than in 2019. In 2020, maximum occurrence and abundance of *R. padi* and *S. avenae* populations occurred at the mid-December and at the end-February sampling periods respectively (Figure 4.3a, 4.3b).

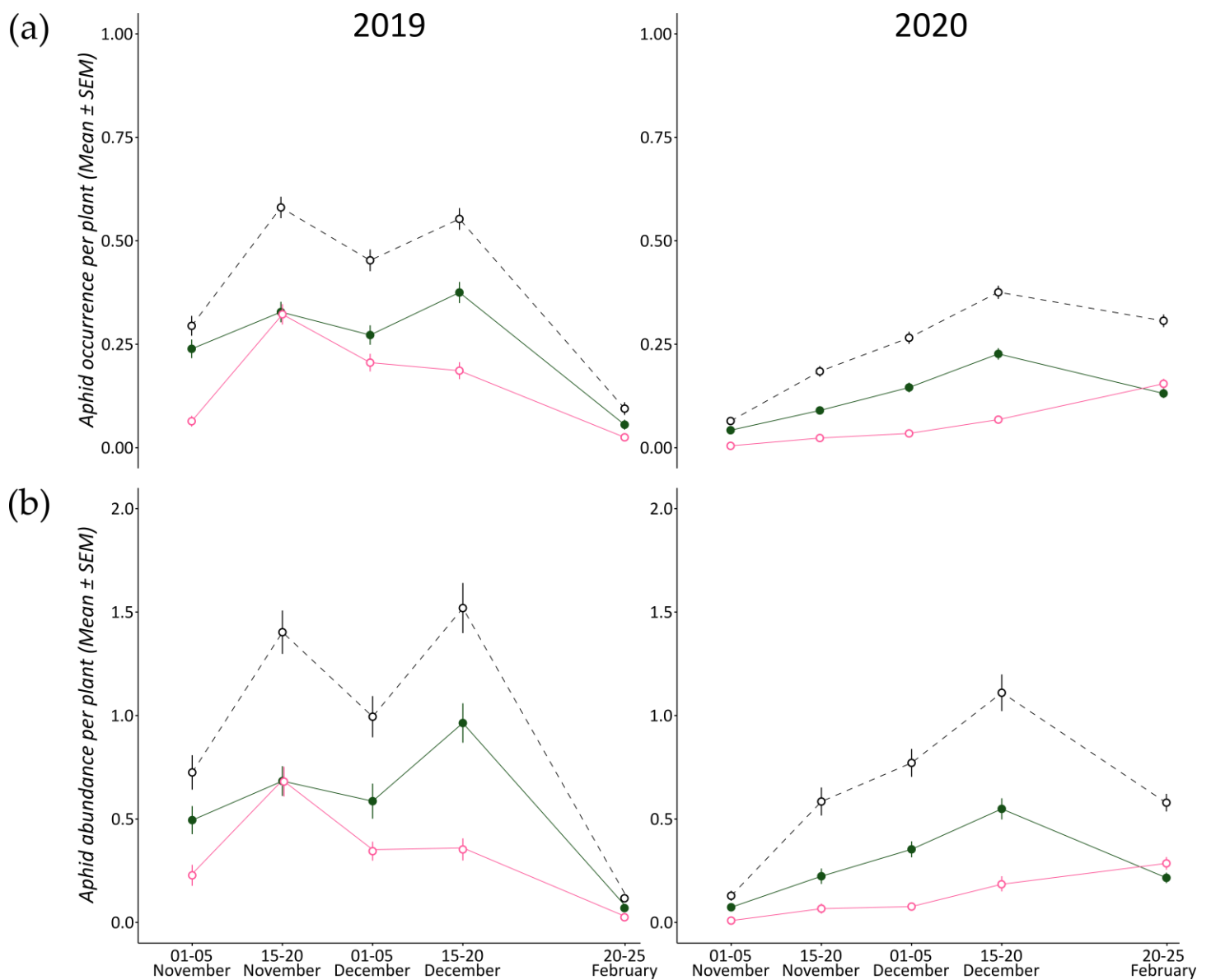


Figure 4.3. Monitoring of aphid populations on barley plants in autumn-winter. (a) Occurrence and **(b)** Abundance (Mean ± SEM) of: All cereal aphids species (—○—), *Rhopalosiphum padi* (—●—), *Sitobion avenae* (—○—). Per sampling period: n=360 plants observed in 2019 (only regular sowings) and n=900 plants observed in 2020.

3.1.3. BYDV-PAV occurrence in barley plants

The BYDV-PAV occurrence per plant was almost null at the first sampling period in mid-November in both years and at the second sampling period in mid-December in 2020 (occurrence < 0.01) (Figure 4.4). Then, towards the end of winter, the virus occurrence reached 0.36 per plant in 2019 (sampling at the end of February), which may reflect the contaminations that took place during winter. In 2020, the infection of barley plants was lower, the occurrence being only 0.10 per plant in the sampling done beginning of April. The additional sampling at the beginning of June 2020, with an occurrence of 0.33 per plant, could correspond to additional infections that took place during spring (Figure 4.4).

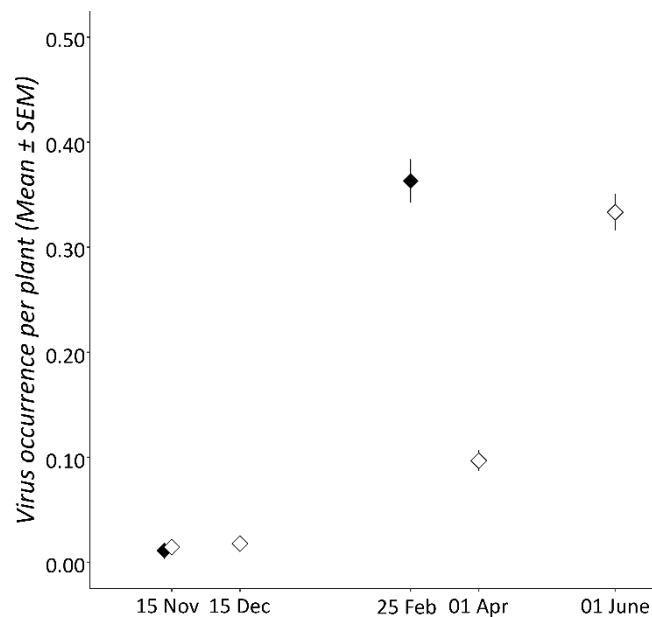


Figure 4.4. Virus occurrence per barley plant in autumn-winter. Mean \pm SEM at the two sampling periods in 2019 (◆, n=360 plants per sampling period, only regular sowings) and the four sampling periods in 2020 (◇, n=900 plants per sampling period).

3.2. Effects of single plant diversification and stacking on the pathosystem cereal aphids-BYDV

3.2.1. Effects on cereal aphids

Plant diversification (variety mixture, intercropping or both combined) had no significant effect on the introduction of alate cereal aphids on monitored plots. Neither the factor *variety*, the factor *clover*, nor the interaction of these two factors (Table 4.5), modified the occurrence or the abundance of alate aphids, regardless of the aphid species considered (Table 4.5, Figure 4.5a and 4.5b).

Intra-specific diversification had no effect on the abundance or occurrence of aphids on barley regardless of the species considered. The factor *variety* was not significant (Table 4.5). Inter-specific diversification also had no effect on aphids, the factor *clover* never being significant. However, the two types of diversification combined had a single significant effect on the occurrence of aphids per plant (all species combined). Indeed, the *variety* \times *clover* interaction was significant for this variable ($p=0.01$) and it can be observed that the aphid occurrence in the mixture/clover treatment was lower than in the mixture treatment. In addition, the occurrence in the mixture/clover treatment was also lower than in the Amistar/clover and Margaux/clover treatments (Figure 4.5c). Thus, the treatment combining the variety mixture and the clover had a lower aphid occurrence than all other treatments. This interaction effect was not observed for *R. padi* and *S. avenae* occurrence, nor on the abundance of cereal aphids (Table 4.5, Figure 4.5c and 4.5d).

3.2.2. Effects on BYDV-PAV

Plant diversification had no effect on BYDV-PAV occurrence, regardless of the type of plant diversification (Figure 4.5e). Neither *variety*, nor *clover* modified significantly the virus occurrence. However, the interaction of the two indicated a trend, but not statistically significant ($p=0.07$, Table 4.5).

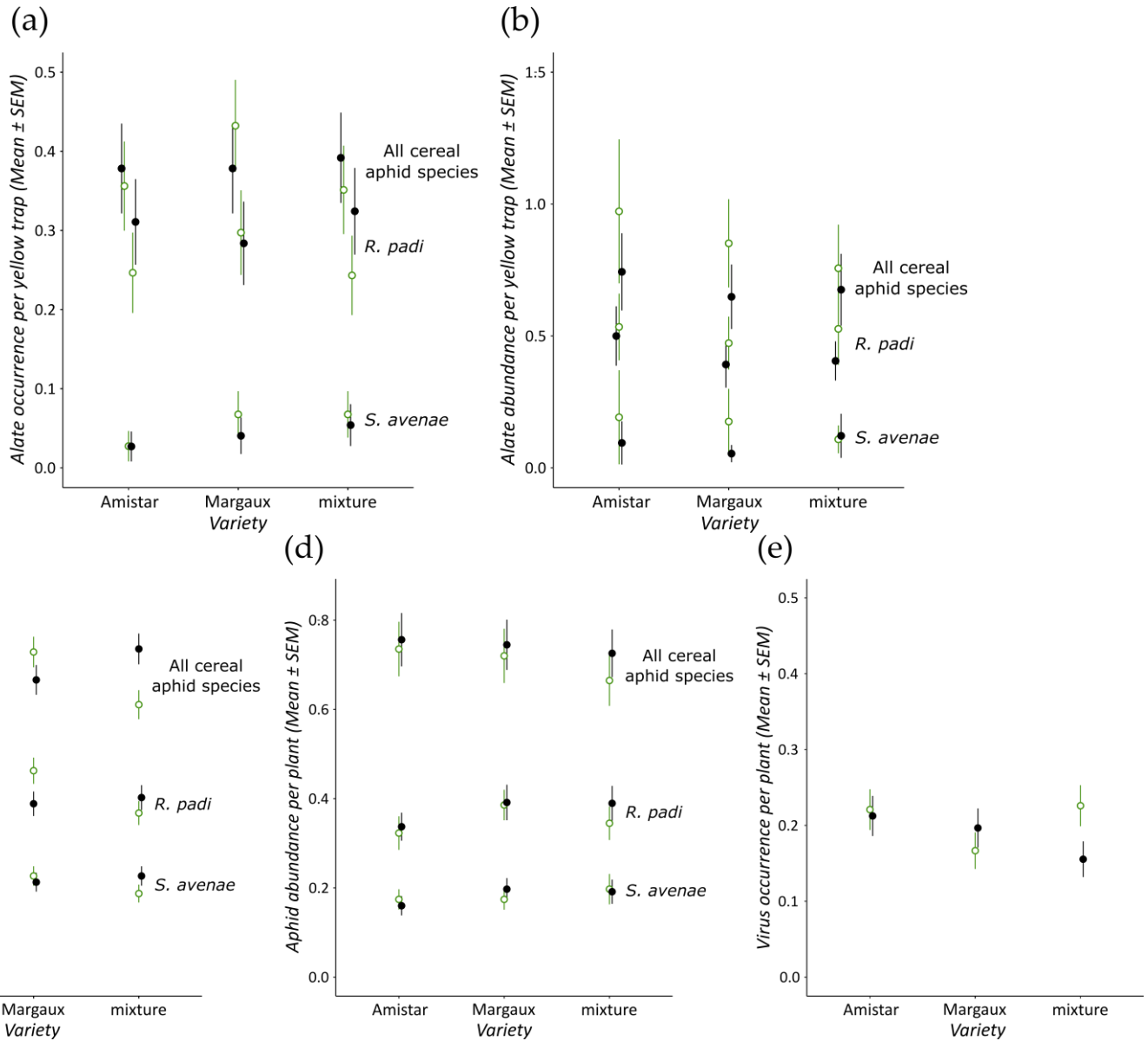


Figure 4.5. Cereal aphid populations and BYDV occurrence in autumn-winter regarding variety mixture or sole variety and barley intercropped or not with clover. (a) Occurrence and (b) Abundance of alate cereal aphids per yellow water trap (Mean ± SEM) (n=74 yellow traps per treatment). (c) Occurrence and (d) Abundance of cereal aphids per barley plant (Mean ± SEM) (n=1320 plants per treatment). (e) Occurrence of BYDV per barley plant (Mean ± SEM) (n=240 plants per treatment). Treatment with clover (○); Treatment without clover (●).

Table 4.5. Statistical test results on the effect of two factors and their interaction on aphid populations and BYDV occurrence in autumn-winter.

Variable tested	Species	Without clover *			With clover *			p-value **			R ² ***		
		Amistar	Margaux	mixture	Amistar	Margaux	mixture	V	C	V × C	R ² m	R ² c	
Alate aphids	occurrence per trap	All cereal aphid species	0.38 ± 0.06	0.38 ± 0.06	0.39 ± 0.06	0.36 ± 0.06	0.43 ± 0.06	0.35 ± 0.06	0.72	0.98	0.61	0.002	0.32
		<i>R. padi</i>	0.31 ± 0.05	0.28 ± 0.05	0.32 ± 0.06	0.25 ± 0.05	0.30 ± 0.05	0.24 ± 0.05	0.97	0.25	0.55	0.004	0.35
		<i>S. avenae</i>	0.03 ± 0.02	0.04 ± 0.02	0.05 ± 0.03	0.03 ± 0.02	0.07 ± 0.03	0.07 ± 0.03	/	/	/	/	/
	abundance per trap	All cereal aphid species	0.74 ± 0.15	0.65 ± 0.12	0.68 ± 0.14	0.97 ± 0.27	0.85 ± 0.17	0.76 ± 0.17	0.53	0.09	0.79	0.006	0.39
		<i>R. padi</i>	0.50 ± 0.11	0.39 ± 0.09	0.41 ± 0.07	0.53 ± 0.13	0.47 ± 0.10	0.53 ± 0.14	0.66	0.28	0.89	0.003	0.37
		<i>S. avenae</i>	0.09 ± 0.08	0.05 ± 0.03	0.12 ± 0.08	0.19 ± 0.18	0.18 ± 0.12	0.11 ± 0.05	/	/	/	/	/
Aphids on barley plants	occurrence per plant	All cereal aphid species	0.29 ± 0.01	0.27 ± 0.01	0.30 ± 0.01	0.28 ± 0.01	0.30 ± 0.01	0.25 ± 0.01	0.72	0.36	0.01	0.0003	0.17
		<i>R. padi</i>	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.19 ± 0.01	0.15 ± 0.01	0.17	0.97	0.06	0.001	0.14
		<i>S. avenae</i>	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.71	0.82	0.32	0.0003	0.27
	abundance per plant	All cereal aphid species	0.76 ± 0.06	0.74 ± 0.06	0.73 ± 0.05	0.74 ± 0.06	0.72 ± 0.06	0.66 ± 0.06	0.41	0.12	0.54	0.0006	0.17
		<i>R. padi</i>	0.34 ± 0.03	0.39 ± 0.04	0.39 ± 0.04	0.32 ± 0.04	0.39 ± 0.03	0.34 ± 0.04	0.06	0.15	0.56	0.001	0.14
		<i>S. avenae</i>	0.16 ± 0.02	0.20 ± 0.02	0.19 ± 0.03	0.17 ± 0.02	0.17 ± 0.02	0.20 ± 0.03	0.11	0.97	0.33	0.0007	0.15
Virus occurrence per plant	BYDV-PAV	0.21 ± 0.03	0.20 ± 0.03	0.16 ± 0.02	0.22 ± 0.03	0.17 ± 0.02	0.23 ± 0.03	0.28	0.38	0.07	0.004	0.33	

V: factor variety (Amistar, Margaux, mixture); C: factor clover (without or with clover); V × C: interaction between the factor variety and the factor clover.

* Mean ± SEM of each of the 6 treatments tested in the field experiment.

** p-values from GLMM models. P-value < 0.05 is written in bold.

*** R²m: marginal R-squared value considering only the variance explained by the fixed effects; R²c: conditional R-squared value considering the variance explained by both fixed and random effects.

3.3. Crop development during the autumn-winter period

At the first sampling period of 2020, in early November, barley was at the 1-leaf stage and covered 8 times more soil than clover reaching only the cotyledon stage. Then, when both crops developed, this difference in soil covering decreased, barley covering on average 4 times more soil than clover from the second sampling period (09/11/20) to the last sampling period of December (14/12/20). At the end of the winter (sampling period of February 2021), the clover cover was unchanged (around 10% of ground cover) whereas barley, at full tillering stage, covered more than 60% of ground (Table 4.6, Figure 4.6a).

During the entire monitoring period from November to end-December, barley was on average 4 times taller than *T. alexandrinum*, 7 times taller than *T. incarnatum* and 16 times taller than *T. repens*. At the end of the winter, mid-February, the clover has developed in height, and the differences between barley and clover was reduced: barley was 2 times taller than *T. alexandrinum*, 4 times taller than *T. incarnatum*, and 9 times taller than *T. repens* (Table 4.6, Figure 4.6b).

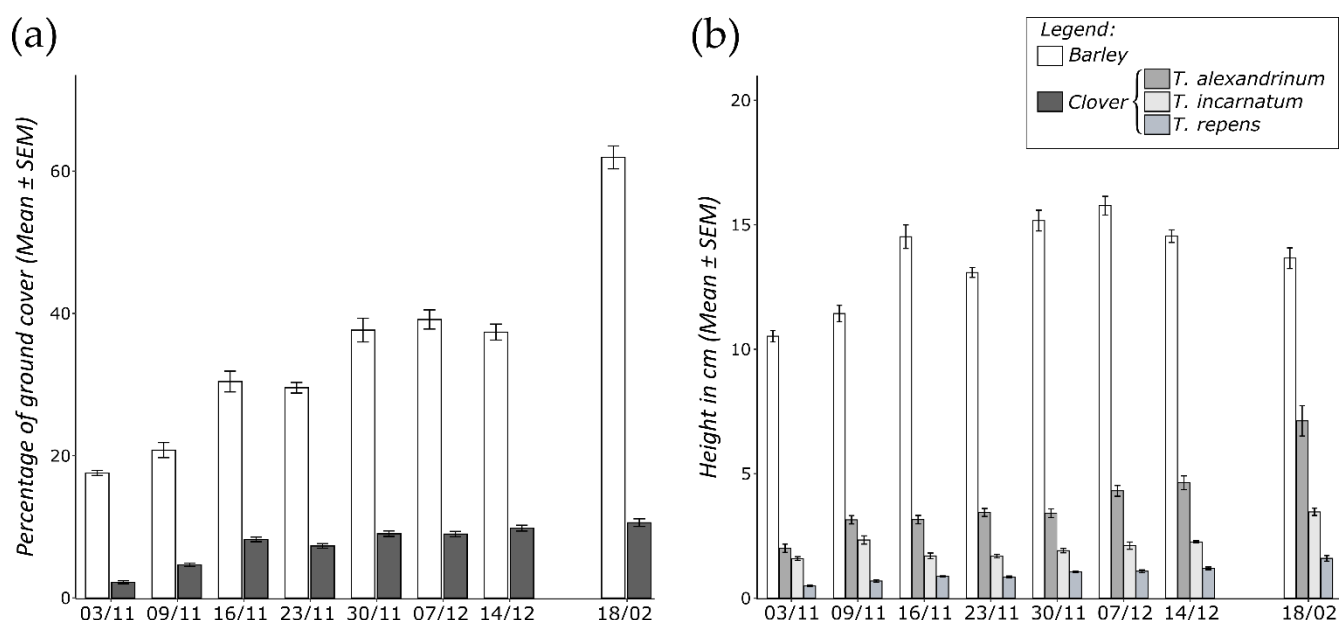


Figure 4.6. Development of barley and clover in autumn-winter.(a) Ground cover and (b) height of barley and clovers (Mean \pm SEM) at the height sampling dates in 2020 (n=90 quadrats and n=45 quadrats for treatment without and with clover respectively).

Table 4.6. Growing stage of barley and clover in autumn-winter.

Sampling period	Growing stage			
	Barley	Clover		
		<i>T. alexandrinum</i>	<i>T. incarnatum</i>	<i>T. repens</i>
03/11/20	1-leaf	cotyledon	cotyledon	cotyledon
09/11/20	2-leaf	1-leaf	1-leaf	cotyledon
16/11/20	3-leaf	2-leaf	1-leaf	1-leaf
23/11/20	1 st tiller	2-leaf	2-leaf	1-leaf
30/11/20	1 st tiller	2-leaf	2-leaf	1-leaf
07/12/20	1 st /2 nd tiller	2-leaf	2-leaf	1-leaf
14/12/20	2 nd tiller	3-leaf	2-leaf	1-leaf
18/02/21	Full tillering	4-leaf	5-leaf	3-leaf

4. Discussion

Cereal aphids, vectors of BYDV, can infest winter cereal crops as soon as they emerge and transmit and spread the virus during winter. In a context of global warming, reduction of pesticide availability and use, and current development of organic farming, also supported by European policy, it is necessary to find new strategies to reduce cereal aphid populations in winter and BYDV incidence. In this study, intra-specific and inter-specific plant diversification, and their combination, were tested to determine their potential to decrease aphid populations (introduction of alate aphids and spread of wingless aphids on cereal plants) and to limit the incidence of BYDV.

4.1. A lack of efficiency of intra-field plant diversification in autumn-winter on the pathosystem

Alate aphids are involved at the beginning of the epidemiological cycle of BYDV in cereal fields. During the two years of the field experiment, alate cereal aphids, represented principally by *R. padi* and *S. avenae* species, mainly arrived in the field during the first fortnight of November, which is correlated with the maximum temperatures period during the autumn and winter sampling periods. The presence of clover did not reduce the alate aphid populations arriving in the field, unlike previous results on alate arrival in intercropped field. Alate aphids are attracted by the soil/plant contrast (Kennedy *et al.*, 1961). In an intercropping systems this ratio is reduced due to the development of the companion plant, resulting in a decrease in aphid landing in the field (A'Brook, 1968; Halbert and Irwin, 1981). In our study, the flight period of alate aphids occurred when the clover was not yet well developed and covered only 5% of the ground. This very low coverage could explain the absence of visual disturbance of alate aphids and therefore an unaltered landing in the intercropping system. In addition, the alate aphid can perform inappropriate landings on the non-host plant, according to the appropriate/inappropriate landing theory of Finch and Collier (2000). In the present study, the very low height of the clover at alate arrival, well below the barley plants in the herbaceous layer, might be insufficient to cause inappropriate landings (less than 3 cm high versus 12 cm, for the clover and barley respectively).

Wingless aphids then occur, in the second step of the epidemiological cycle of BYDV within the field. Aphids monitored on barley plants, mainly wingless, were present during the whole monitoring period from November to December, and during the last monitoring period in February. Thus, populations were probably maintained by parthenogenesis throughout the winter and could be able to spread BYDV within the field. The practice of inter-specific diversification tested in the field experiment did not reduce cereal aphid populations compared to pure barley crop treatments. This result contrast with laboratory observations that indicated a decrease in cereal aphid population in the presence of clover (Grauby *et al.*, 2022). However, this laboratory study involved more developed and dense clover plants than in this field experiment. Effectively, the plants of the mono-specific clover cover measured 6 cm high and reached a 2 leaves-stage. In the present field experiment, when wingless aphid populations reached a maximum (first fortnight of December), the multi species clover cover was composed of smaller plants (4 cm being the highest species, 2 cm and 1 cm high for the two others), reaching only a 1 or 2-leaves stage and covered merely 9% of the ground. Furthermore, in the laboratory study conducted by Mansion-Vaquié *et al.* (2020), where aphid population was also decreased with intercropping, the clover plant density was three times higher than the one used in this field experiment (the density ratio clover : cereal being 3:1 and 1:1 respectively). The density chosen in our study being the one commonly used by farmers in cereal-clover simultaneous sowing in autumn.

These major differences detected in clover canopy development (plant density, development stage and height), could lead to higher clover biomasses in laboratory than in the field experiment. The different mechanisms of aphid disturbance by a non-host plant, being olfactory, visual or physical (Perrin and Phillips, 1978; Finch and Collier, 2000; Randlkofer *et al.*, 2010; Xie *et al.*, 2012; Ninkovic *et al.*, 2013), could be increased in presence of a higher non-host plant biomass. With a poorly developed clover cover, as the one in the field experiment, it is unlikely that these disturbances will occur significantly. In addition, other parameters may explain the different effectiveness of the practice in the laboratory and in the field such as the aphid pressure or the development stage of the barley.

Regarding intra-specific plant diversification, the barley variety mixture did not reduce cereal aphid populations in cereals either, although laboratory results showed promising effects (Pettersson *et al.*, 1999; Ninkovic *et al.*, 2002; Shoffner and Tooker, 2013; Dahlin *et al.*, 2018). The underlying plant-plant communication mechanism via volatiles may be disturbed under field conditions, via a dilution of volatiles in the olfactory landscape, decreasing effects on aphids. Other parameters such as aphid pressure could explain the lack of effectiveness of this practice in the present study.

In contrast to single plant diversification treatments, the stacking of intra- and inter-specific plant diversity levels led to a significant effect in our field experiment by decreasing the occurrence of cereal aphids (all species grouped together). This result could not be confirmed by Mansion-Vaquié *et al.* (2019) which tested also stacking with wheat variety mixtures combined with white clover intercropping. However, although the sowing of their field experiment was carried out at the same time as our study in autumn, the aphid monitoring was carried out in spring/summer, which makes a comparison of the results delicate. Further studies should be conducted in autumn-winter to confirm or refute our results.

Concerning the BYDV, the different types of diversification, did not significantly decrease the incidence of the virus. There was even an inverse tendency with higher virus occurrence in the variety mixture/clover stacking treatment than in the other single treatments.

4.2. Possibilities to improve the practice by increasing the biomass of the companion plant

As discussed previously, the laboratory results are so far convergent concerning the potential of inter-specific plant diversification to reduce cereal aphid populations. It is therefore legitimate to expect that this effect can be replicate in the field, on condition that the right cover development parameters are taken into account. The relative biomass of the cover crop compared to the cash crop seems to be an important parameter in the bottom-up regulation of aphids. By adjusting this variable, the disruption of aphids in the several stages of the epidemiological cycle of BYDV in winter could be more optimised. The biomass of the cover crop could for example be adjusted through the sowing date and sowing density, the spatial or temporal arrangement of the cover and cash crops or the choice of the cover crop species. However, such parameters must remain coherent and relevant to farmers' agronomic and economic realities such as for example, yield loss due to crop competition, YDD risk, and economic investment in seeds.

In our case, the clover was sown simultaneously with the barley mid-October resulting in a limited development of the clover cover. An earlier sowing would have allowed the clover to be more developed but, at the same time, it would have increased the YDD risk for the cereal due to a longer exposure to alate aphid flight (Poehling *et al.*, 2007). The sowing density of the clover,

could also have been increased and with it the probability for aphids to encounter a non-host plant. However, the sowing density used in this field experiment (1 clover plant for 1 barley plant) showed already averaged yield losses of about 15% compared to non-intercropped treatments (Grauby *et al.*, not published). Concerning the spatial arrangement of crops, the mixed intercropping used in this study was, according to the farmers, the simplest one to manage. Other spatial arrangements such strip or row intercropping (Andrews and Kassam, 1976) could have been considered in regard with their potential to disrupt aphids by increasing the physical barrier between the barley rows. In the same idea, different temporal arrangements, e.g. “asynchronous intercropping” (Gardarin *et al.*, 2022) could increase the relative biomass of the cover crop on autumn cereal fields. But in these cases, the agronomic challenges of the cover crop/cash crop association and the technical constraints for farmers would have been highly intensified.

Finally, the choice of the cover crop species is an important lever to activate for an increased biomass on the field. For example, cover crop plants with a rapid germination, development and a well-developed aerial architecture from autumn-winter could be favoured in their use in a simultaneously intercropping with a cash crop (Lorin *et al.*, 2015, Singer *et al.*, 2006). In our experiment, a mixture of three clover species was used and *T. alexandrinum* was the fastest clover to grow and cover the soil. A *T. alexandrinum* mono-specific cover could therefore have resulted in a higher cover crop biomass and potentially a more disruptive effect on cereal aphids in autumn. But in this case, the competition with the young barley plants might have been stronger.

To conclude, it appears crucial to carefully consider the trade-offs between the benefits linked to the decrease of aphid populations and YDD through a non-pesticide based practice and the drawbacks in terms of within crop competition and associated yield losses, of technical constraints and economic costs.

Acknowledgements: We thank Anthony Roume for his help in setting up the field experiments, Adeline Cadiergues, Violaine Herisson and Lena Jego for their technical assistance in the field monitoring, and Pierre Deroo and Lena Jego for their assistance in species identification of alate aphids. We further thank Vincent Tolon for his help in the statistical analyses of the data. We are very grateful to the farmers that permitted to carry out this on-farm research on their fields.

Funding: This work was supported by the European Union’s Horizon 2020 Research and Innovation programme, under Grant Agreement no. 773554 (EcoStack).

Part 2. Effect of intra-field plant diversification on aphid natural enemies, at the beginning of a winter cereal growing cycle

In the autumn-winter period, aphid foliar enemies, as well as ground-dwelling arthropods, were also monitored in the field experiment. The monitoring methods, the results and a discussion are presented in this supplementary data part.

1. Materials and methods

1.1. Aphid foliar enemies

Foliar enemies of aphids were counted during the aphid monitoring, on the same 30 plants per treatment (Figure 4.7). For the foliar predators, their development stage was recorded (egg, larva, pupa or adult). For the parasitoids, mummy abundance (i.e. last parasitoid larval instar) was monitored, as they are easily countable in the field. For each mummy, the emergence status of the parasitoid was monitored through the observation of emergence hole. Parasitism rate was calculated as the ratio of mummies to total aphids (including mummies). The mummies were collected and transferred to the laboratory. The emergence of parasitoids was regularly monitored.

The foliar enemies monitoring, as the aphid monitoring, started when the first barley leaf was emerged and was carried out every two weeks until mid-December (i.e. for regular sowing monitoring started two weeks after sowing for a total of four sampling periods, for late sowing it started three weeks after sowing for a total of two sampling periods). An additional sampling period took place at the end of February.

1.2. Ground-dwelling arthropods

Ground-dwelling arthropods were monitored using three pitfall traps placed in the central line of each treatment (Figure 4.7). For more details on the traps see the general material and method (chapter 2). The traps were opened for 48 hours. Ground beetles and spiders were identified at the species and at the family level, respectively, while rove beetles were counted without further identification. Two trapping periods were set up in autumn-winter (mid-November and end-February).

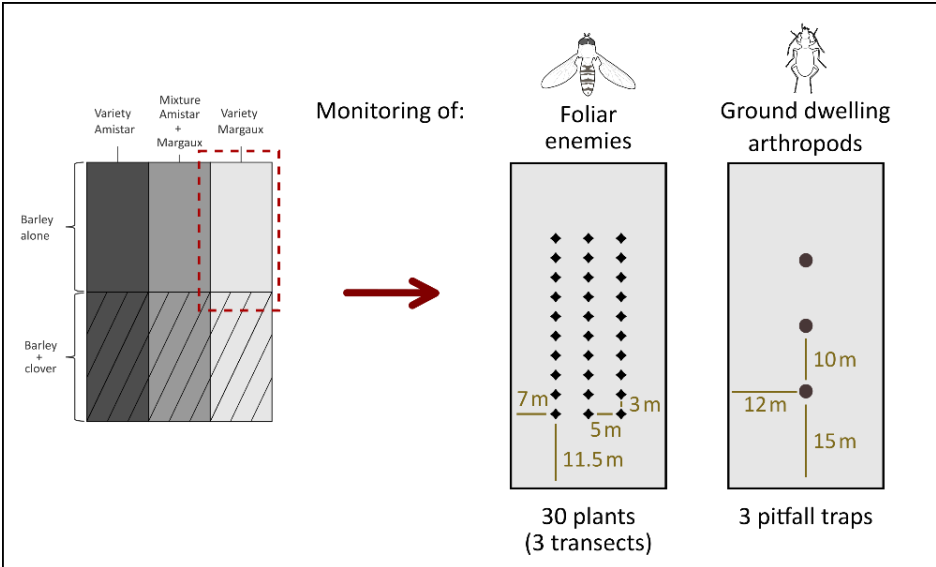


Figure 4.7. Monitoring protocols implemented in the field experiment in autumn-winter.

1.3. Statistical analyses

Activity-density of ground beetles, spiders and rove beetles were analysed using GLMM (see chapter 4 part 1, the same models were used). A negative binomial link was used.

Variable tested \sim variety + clover + variety \times clover + (1 | sampling period) + (1 | site)

Concerning aphid foliar enemies, their low number did not permit statistical analyses to be carried out (Table 4.7).

2. Results

2.1. Aphid foliar enemies in autumn-winter

Except for mummies and hoverflies, no aphid foliar enemies (ladybirds, lacewings, *etc.*) were observed on the sampled plants in autumn-winter (Table 4.7).

Table 4.7. Aphid foliar enemies observed on barley plants in autumn-winter in the two years of monitoring.

Aphid foliar enemy	Stage	Year *	
		2019	2020
Mummy	Unhatched	77	4
	Hatched	0	2
Hoverfly	unhatched egg	0	42
	Larva	0	4
	Total	77	52

* Total number of individuals per taxon for each year of monitoring (n=3420 plants observed in 2019 and n=4500 plants observed in 2020).

2.1.1. Parasitoid

In autumn-winter 2019, from the 3420 plants sampled, a total of 77 unhatched mummies (without an emergence hole) were counted (Table 4.7). On these same plants, a total of 1817 aphids were counted (see chapter 4 part 1), the overall parasitism rate was thus 4%. At the first two sampling periods, counts were null. Then, at the beginning of December 9 mummies were counted and a maximum was reached at the mid-December sampling period (51 mummies) (Figure 4.8a). At this same sampling period, a total of 544 aphids was counted, the parasitism rate was thus 8%. At the end-February sampling period, 15 mummies were counted.

From the 77 mummies transferred to the laboratory, 59 parasitoids emerged. 21 emerged within 9 days after being collected from the field. 38 emerged after 4 to 21 days (no precise dating due to the laboratory Christmas closure). 9 did not emerge (8 collected in the mid-December sampling period and 1 collected in the late February sampling period). 9 mummies were damaged or lost after the field collection. Thus the rate of emergence within 3 weeks after being collected was 87%.

In autumn-winter 2020, from the 4500 plants sampled, a total of 6 mummies were observed (4 unhatched and 2 hatched) (Table 4.7, Figure 4.8b). On these same plants, a total of 2851 aphids were counted (see chapter 4 part 1). Thus the overall parasitism rate was 0.2%.

2.1.2. Hoverfly

In autumn-winter 2019, no hoverfly was observed (Table 4.7). In autumn-winter 2020, from the 4500 plants sampled, 42 unhatched hoverfly eggs and 4 hoverfly larvae were observed (Table 4.7). A maximum of 27 hoverfly eggs was reached at the first sampling period (beginning of November), then 12 eggs were counted mid-November. Then, counts dropped with 1 and 2 eggs observed at the sampling period of beginning and mid-December respectively (Figure 4.8b).

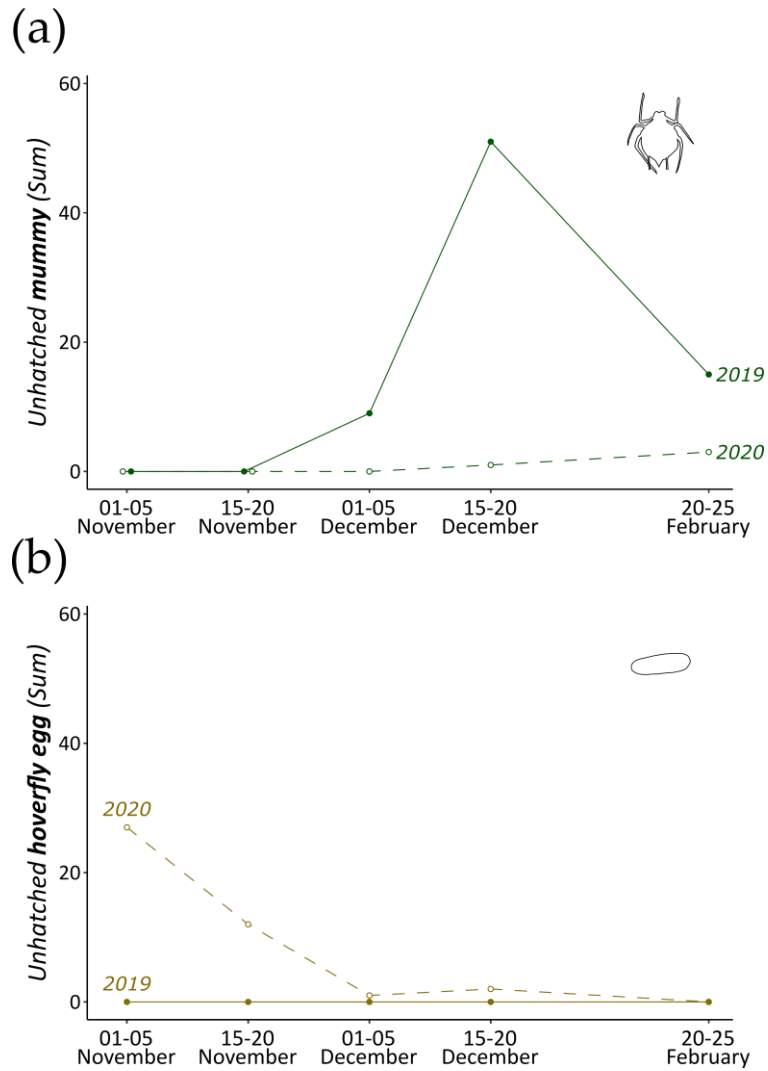


Figure 4.8. Monitoring of aphid foliar enemies on barley plants in autumn-winter. Sum of: **(a)** Unhatched aphid mummy in 2019 (—●—) and in 2020 (- -○- -) and **(b)** Unhatched hoverfly egg in 2019 (—●—) and in 2020 (- -○- -). Per sampling period: n=900 plants observed in 2019 and n=900 plants observed in 2020.

2.2. Ground-dwelling arthropods in autumn-winter

2.2.1. Taxonomic diversity and abundance of ground-dwelling arthropods

Over the two autumn-winter periods studied, the most represented taxon in pitfall trap catches was rove beetles (715 individuals in total), followed by ground beetles (576 individuals) and spiders (352 individuals) (Table 4.8). However, for each taxon, inter-annual abundance variations were observed: 1.4 times more rove beetles and spiders were caught in 2019 than in 2020. Conversely, 1.7 times more ground beetles were caught in 2020 than in 2019 (Table 4.8).

The 576 ground beetles caught during the two years of monitoring belonged to 23 different species. The three main abundant species were *Trechus quadristriatus*, *Nebria salina* and *Harpalus distinguendus*, which together represented 87% of all individuals captured (Table 4.8). The 352 spiders captured belonged to 13 different families. The main family was the Linyphiidae representing 66% of total captures, followed by Lycosidae, which represented almost 19% of captures (Table 4.8).

Table 4.8. Ground-dwelling arthropods collected in pitfall traps in autumn-winter in the two years of monitoring.

Lower level taxon		Year		Total *
		2020	2021	
Rove beetles	Total	419	296	715
Ground beetles	<i>Trechus quadristriatus</i>	141	139	280 (49%)
	<i>Nebria salina</i>	14	112	126 (22%)
	<i>Harpalus distinguendus</i>	33	64	97 (17%)
	<i>Phyla obtusa</i>	1	13	14 (2%)
	<i>Acupalpus meridianus</i>	0	10	10 (2%)
	<i>Harpalus affinis</i>	0	7	7 (1%)
	<i>Pseudoophonus rufipes</i>	1	5	6 (1%)
	Other (16 species)	19	17	36 (6%)
	Total	209	367	576 (100%)
Spiders	Linyphiidae	145	88	233 (66%)
	Lycosidae	31	35	66 (19%)
	Gnaphosidae	22	6	28 (8%)
	Thomisidae	4	5	9 (3%)
	Other (9 families)	6	10	16 (4%)
	Total	208	144	352 (100%)

* Total number of ground-dwelling arthropods per taxon. Relative proportion of the dominant ground beetle species and spider families (i.e. representing at least 1% of the total) counted during the whole monitoring period (n=180 pitfall traps in 2019 and n=180 pitfall traps in 2020).

2.2.2. Temporal dynamic of ground-dwelling arthropods

At the first sampling period in early November 2019, ground beetles and spiders activity-density were comparable (almost two individuals per pitfall trap) while rove beetles activity-density was about twice as high (Figure 4.9). On the same period in 2020 rove beetles and spiders activity-density were close (around one individual per trap) and ground beetles activity-density was much higher (three individuals). At the second monitoring period, late February, the populations of the three taxa were quite low and comparable in 2019 (less than one individual). In 2020, at the same period, the populations of rove beetles were higher than those of ground beetles, and those of spiders.

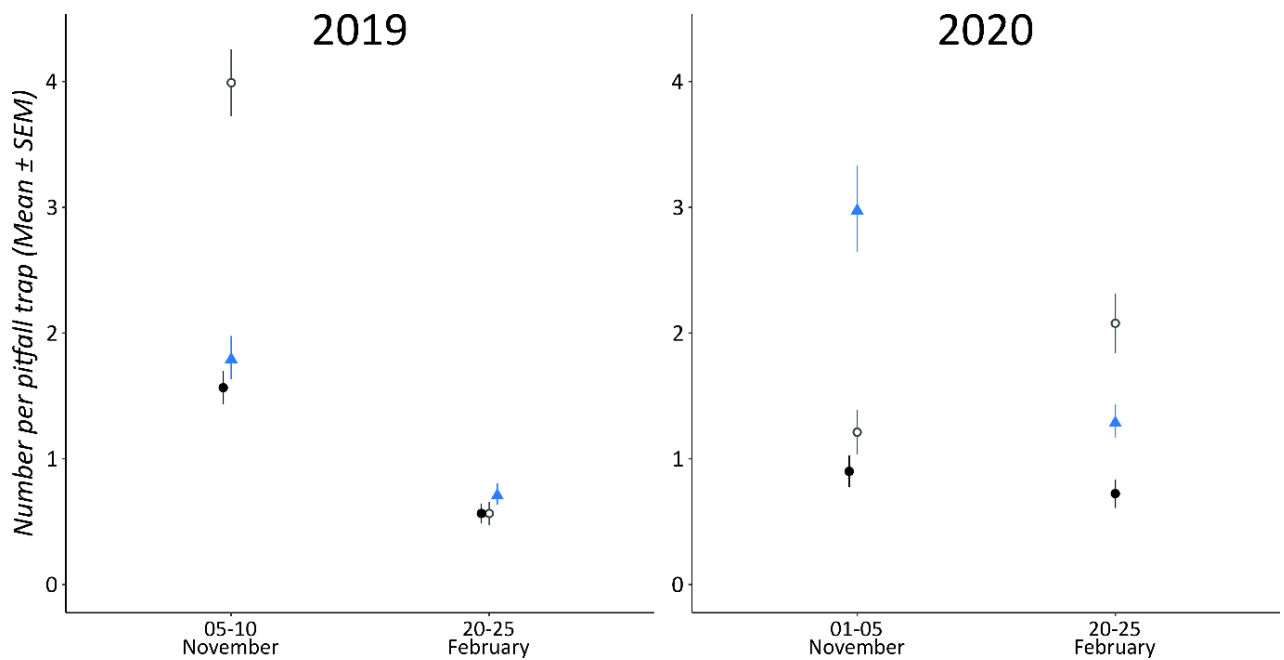


Figure 4.9. Ground-dwelling arthropod activity-density in autumn-winter. Mean \pm SEM per pitfall trap of: Ground beetles (▲), Spiders (●), Rove beetles (○). Per sampling period: n=90 pitfall traps in 2019 and n=90 pitfall traps in 2020.

The species *Trechus quadristriatus*, showed different activity-density patterns between the two years of monitoring (Figure 4.10). In 2019, the species activity-density was high at the beginning of November and was almost null at the end of February. In 2020, the species was again active at the beginning of November but it was also active at the end of February. For the species *Nebria salina*, its activity-density was very low in the November 2019 sampling period and null at the end of February. In 2020, the activity-density of the species was quite high in early November and again null in late February. Finally, the species *Harpalus distinguendus* showed different patterns between the two years. Its activity-density was almost null at the beginning of November 2019, then increased at the end of February. In 2020, its activity-density was higher at the beginning of November, then reduced by half at the end of February.

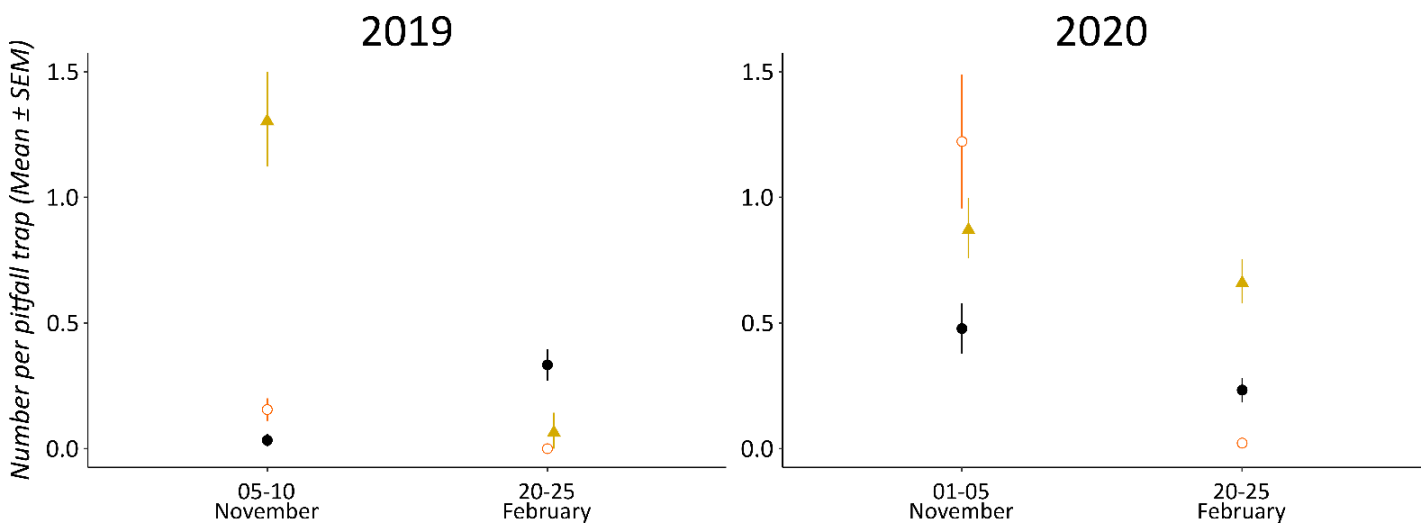


Figure 4.10. Ground-beetle activity-density in autumn-winter. Mean \pm SEM per pitfall trap of: *Trechus quadristriatus* (▲), *Nebria salina* (○), *Harpalus distinguendus* (●). Per sampling period: n=90 pitfall traps in 2019 and n=90 pitfall traps in 2020.

2.2.3. Effects of plant diversification on ground-dwelling arthropods

Plant diversification had no significant effect on ground-dwelling arthropods activity-density in autumn-winter, regardless of the taxon considered: ground beetles (Figure 4.11a), spiders (Figure 4.11b) or rove beetles (Figure 4.11c). Neither the factor *variety*, the factor *clover*, nor the interaction of these two factors, modified the activity-density of ground-dwelling arthropods (Table 4.9).

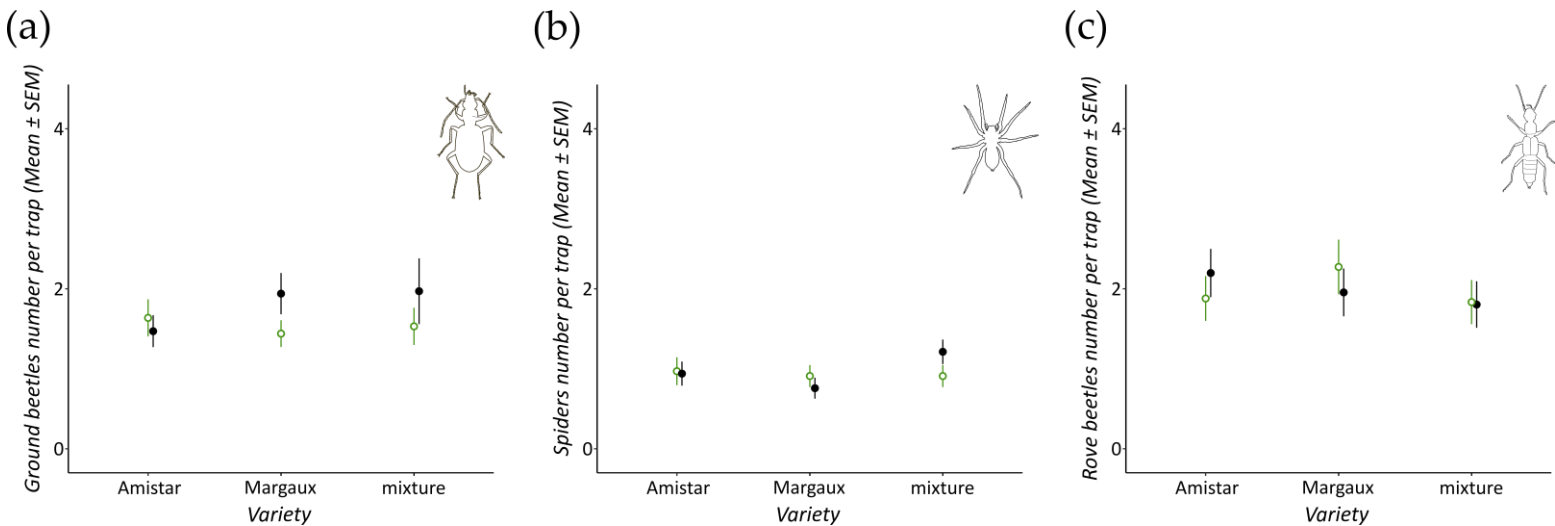


Figure 4.11. Activity-density of ground-dwelling arthropods in autumn-winter regarding variety mixture or sole variety, and barley intercropped or not with clover. Mean \pm SEM per pitfall trap of: (a) All spider families; (b) All ground beetle species and (c) Rove beetles. Per treatment: n=30 pitfall traps in 2019 and n=30 in 2020. Treatment with clover (○); Treatment without clover (●).

Table 4.9. Statistical test results on the effect of two factors and their interactions on ground-dwelling arthropod activity-density in autumn-winter.

Variable	Without clover *			With clover *			p-value **			R ² ***	
	Amistar	Margaux	mixture	Amistar	Margaux	mixture	V	C	V × C	R ² m	R ² c
Ground beetles	1.47 \pm 0.20	1.94 \pm 0.26	1.97 \pm 0.41	1.64 \pm 0.23	1.44 \pm 0.17	1.53 \pm 0.23	0.62	0.18	0.28	0.005	0.40
Spiders	0.94 \pm 0.15	0.76 \pm 0.13	1.21 \pm 0.15	0.97 \pm 0.17	0.91 \pm 0.14	0.91 \pm 0.14	0.25	0.73	0.19	0.006	0.23
Rove beetles	1.88 \pm 0.28	2.27 \pm 0.34	1.83 \pm 0.28	2.20 \pm 0.30	1.95 \pm 0.30	1.80 \pm 0.29	0.28	0.85	0.32	0.004	0.53

V: factor variety (Amistar, Margaux, mixture); C: factor clover (without or with clover); V × C: interaction between the factor variety and the factor clover.

* Mean \pm SEM of each of the 6 treatments tested in the field experiment.

** p-values from GLMM models.

*** R²m: marginal R-squared value considering only the variance explained by the fixed effects; R²c: conditional R-squared value considering the variance explained by both fixed and random effects.

3. Discussion

3.1. Aphids' foliar enemies activity in autumn-winter

Mummies were significantly present in autumn-winter one year on two, in 2019. This year, the maximum number of mummies occurred in mid-December, for an average parasitism of 8%. However, the real parasitism is certainly more important than this observed one. In the field, only the advanced parasitized stages were identified (visible mummies) and the early stages were simply counted as unparasitized aphids. It would have been necessary to bring all aphids counted to the laboratory and count the number of mummies formed after a few days, which would have been extremely time consuming. 87% of the mummies emerged within 3 weeks after their transfer to the laboratory (the 3 weeks being probably overestimated due to laboratory closure). These 87% should represent non-diapausing individuals, remaining active during the winter and parasitizing aphids, which continue to reproduce by parthenogenesis (see chapter 4 part 1). The remaining 13% were not dissected. This would have allowed us to determine the proportion of diapausing (visible parasitoid prepupae) or dead individuals (Tougeron, 2017).

Concerning hoverflies, eggs were observed in early and mid-November 2020. Adults were therefore probably active until this period, depositing eggs on aphid-infested plants. Thus, hoverflies may contribute to regulate the first aphid infestations early in the autumn (Raymond *et al.*, 2014). Then, during the following sampling periods, no hoverflies were observed. Thus, they probably entered into diapause. Hoverflies can overwinter as adults (Sarhou *et al.*, 2005) or as larvae or pupae (Dusek and Laska, 1974; Sarhou *et al.*, 2005) in the soil (Sarhou *et al.*, 2005; Bokina, 2012). Raymond *et al.* (2014) showed that aphidophagous hoverfly species preferentially overwinter in agricultural fields (unlike non-aphidophagous hoverflies which tend to overwinter in semi-natural areas or on field edges). These species of aphidophagous hoverflies, emerging in the spring, could then regulate aphid populations precociously in cereal fields. Raymond *et al.* (2014) also observed a greater attractiveness of diapausing aphidophagous hoverfly species for certain crops in autumn, potentially related to a more developed vegetation.

In our study, we observed an activity of hoverflies in early autumn and of parasitoids in winter, which represents an interesting complementarity for the biological control of cereal aphids. The clover cover was poorly developed in autumn, and non-flowering, which is not particularly attractive for increasing their populations. A flowering cover, and its source of pollen and nectar, could potentially attract adult hoverflies and parasitoids, laying eggs in aphid colonies or parasiting aphids, respectively. A more developed cover may also increase the number of overwintering hoverflies within the field (Raymond *et al.*, 2014).

3.2. Ground-dwelling arthropods activity in autumn-winter

Ground-dwelling arthropods, are mostly considered to overwinter within semi-natural habitats surrounding monocultures (Sotherton, 1984, Sotherton 1985; Collins *et al.*, 2003; Frank and Reichhart, 2004; Thomas *et al.*, 1992; Woodcock *et al.*, 2005) and to colonise arable crops after their hibernation. Winter-active communities are poorly studied so far (Damien, 2018). In the present study, ground-dwelling predators were sampled on two dates, early November and late February. It is therefore not possible to determine precisely which taxa/species were active throughout the autumn-winter. However, these two dates allow us to discuss their presence at two specific periods, in mid-autumn and in the second half of winter.

Rove beetles were the most abundantly caught taxon in the study. In 2019 at the beginning of November, it was the taxon with the highest activity-density, and also at the end of February in 2020. Most rove beetle species are univoltine and overwinter as adults (Andersen 1982; Balog and Markó 2007). Thus, the individuals caught may have been individuals that had not yet started their overwintering (early November) and those that had just emerged from overwintering (late February), or they may have been non-overwintering individuals. Similarly, with spiders, the third most captured taxon, active individuals were caught on both monitoring dates. Studies have shown that some spider species remain active in winter and actively hunt (Marc and Canard, 1997; Miliczky *et al.*, 2008; Korenko and Pekar, 2010).

Ground beetles were the second most caught taxon in the study. *Trechus quadristriatus* and *Nebria salina*, the dominant species caught, are autumn breeders (Alford, 1979; Lindroth, 1945), i.e. they reproduce in late summer/autumn and overwinter as larvae in the soil. In the present study, adults of both species were still active during the November sampling period. Both species are predatory (Ribera *et al.*, 2001) and were therefore susceptible to feed. Furthermore, it is important to note that in 2020, the species *Trechus quadristriatus* was still active in the late February sampling period. It is unlikely that these adults had already pupated and emerged from the soil, autumn breeder adults emerging later, in summer. It is more probable that these adults have spent the whole winter without a dormancy, as it may exist with some ground beetles (Thiele, 1977). On this matter, studies found that adults of *Trechus quadristriatus* can survive more than one season (Warner *et al.*, 2003) staying active during the winter and continuing to feed (Alford, 1979). Conversely, the species *Nebria salina* was no longer active in February during the two years of monitoring, probably being still in diapause at that time of the year. Finally, the species *Harpalus distinguendus*, the third dominant species, is a spring breeder (Lindroth, 1945), i.e. it overwinters as an adult and reproduces in spring. Adults overwinter in a state of diapause or quiescence (Thiele, 1977). In the present study, the species was still active in early November 2020, probably before overwintering. It should be noted that the species was still active, at the end of February: these individuals may have come out of quiescence. However, this species of ground beetle is mainly granivorous, and will not be a significant consumer of aphids. The forms of dormancy in ground beetles are diverse (diapause, quiescence), and some individuals probably do not even have dormancy (Thiele, 1977). Our study does not allow a precise assessment of the activity of ground beetles throughout the winter, and their ability to feed on aphids (or other food resources) in order to survive. However, these two monitoring periods provide insight into ground beetles activity in mid-autumn and in the second half of winter.

3.3. No effect of inter-specific plant diversification on ground-dwelling arthropods



Although some ground-dwelling arthropods were active during the two sampling periods, the presence of clover did not increase their populations. The clover cover was poorly developed during the autumn-winter period, it is thus unlikely to act as a shelter or habitat for them and locally increase their populations. A higher biomass in autumn-winter could promote active individuals during this period, providing them more sheltered habitat (from cold, rough weather or predators) and potentially food resources. A dense cover could also allow more individuals to overwinter directly within the field, rather than overwintering in peripheral habitats, and allow an early and rapid crop colonisation in spring (Symondson *et al.*, 2002).

Part 3. Synthesis of the autumn-winter results

		Autumn-Winter		
		 { var Amistar var Margaux	 { var Amistar var Margaux Mixture	
		 intra	 inter	 intra + inter
Virus				
Cereal aphids	All sp.			occurrence mix.C vs. mix occurrence mix.C vs. am.C mar.C
	<i>S. a</i>			
	<i>R. p</i>			
	<i>M. d</i>			
Foliar enemies				
Ground dwelling arthropods	All sp.			
	<i>A. d</i>			
	<i>P. c</i>			
	All fam			
	Lyc			
Lin				

Figure 4.12. Synthesis of the autumn-winter results of the field experiment. Effects of different types of diversification (intra-specific, inter-specific and both combined) on:

Occurrence of the BYDV virus () , abundance/occurrence of alate cereal aphids () , abundance/occurrence of cereal aphids on barley () All sp. = all species combined, *S. a* = *Sitobion avenae*, *R. p* = *Rhopalosiphum padi*, *M. d* = *Metopolophium dirhodum* ; abundance of hoverfly eggs (hatched + unhatched) () ; abundance of mummies (hatched + unhatched) () ; abundance of ground beetles ()

A. d = *Anchomenus dorsalis*, P. c = *Poecilus cupreus*; abundance of spiders () All fam = all families combined, Lyc = Lycosidae, Lin = Linyphiidae; and abundance of rove beetles ().

Plant diversification may increase (↗), decrease (↘) or have no effect (⊘) on abundance/occurrence.

A green or red arrow indicates respectively a positive or negative effect of plant diversification on observed parameters in the sense of biological regulation (i.e. green which decreases the pest and increases its natural enemies and red the opposite).

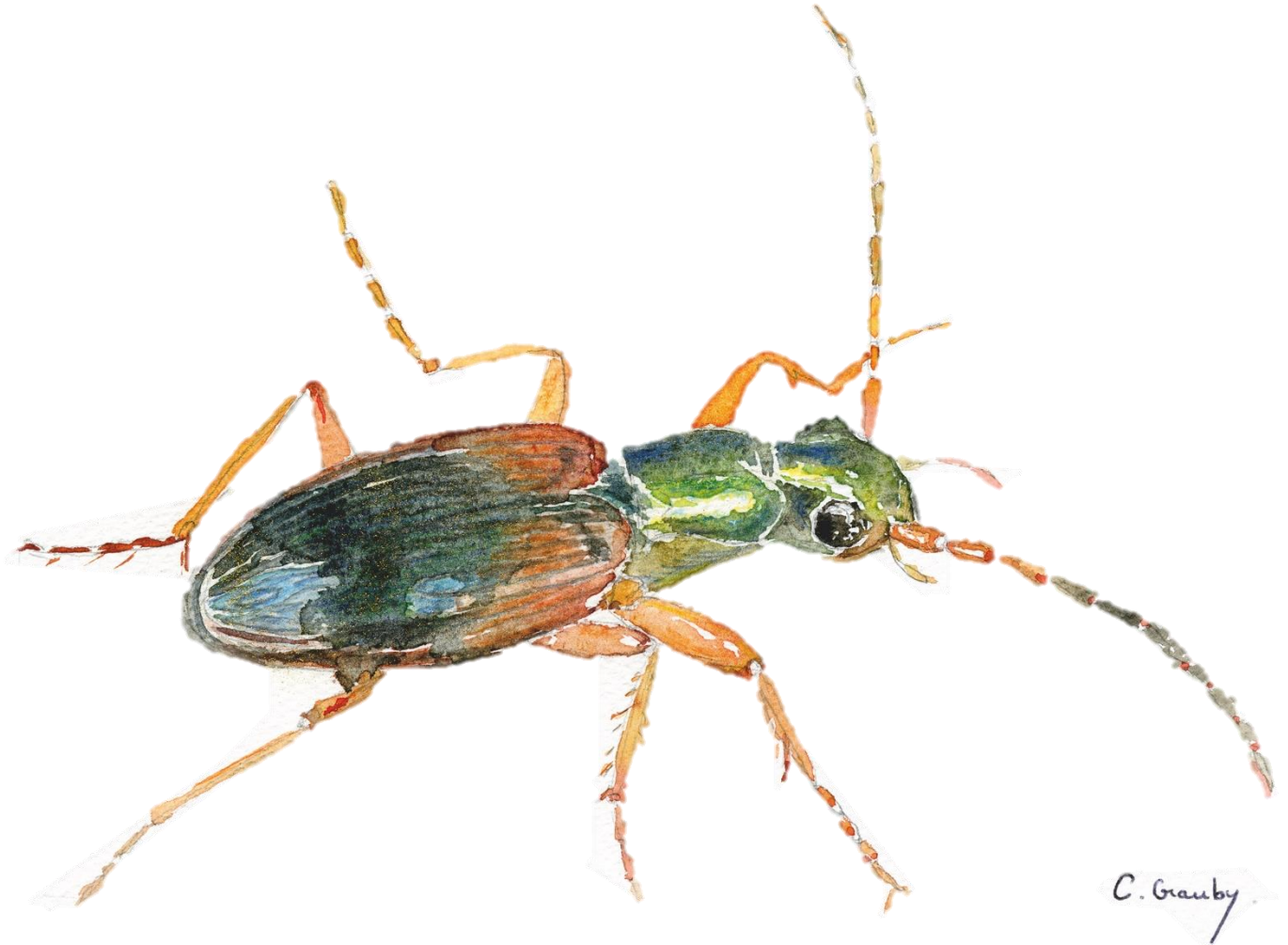
am = Amistar variety, **mar** = Margaux variety, **mix** = mixture.

am.C = Amistar variety intercropped with clover.

NB : " ↘ occurrence mix.C vs. mix " means "decreased occurrence in the mixture/clover modality compared to the mixture variety alone".

CHAPITRE 5

EFFET DE LA DIVERSIFICATION VEGETALE INTRA-PARCELLAIRE SUR LES PUCERONS DES CEREALES ET LEURS ENNEMIS NATURELS AU PRINTEMPS



Aquarelle, *Anchomenus dorsalis*
Christine Grauby

Chapitre 5. Effet de la diversification végétale intra-parcellaire sur les pucerons des céréales et leurs ennemis naturels au printemps

Part 1. Spring effect of intra-field plant diversification on cereal aphids and ground-dwelling arthropods in winter barley crop

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Keywords: Intercropping; cultivar mixture; clover; ground-beetles; spiders; rove beetles

Abstract: Aphids pose serious issues in many plant production systems. Plant diversification in cropping system appears as a promising strategy to reduce insect pest populations on crops and to favour natural enemy populations. Indeed, an inter-specific diversification through plant association can reduce insect pest populations by bottom-up regulation (dilution effect, physical barrier, olfactory disturbance, etc.) and also with top-down regulation (attracting their natural enemies with shelter, food source, etc.). The use of varieties mixture, i.e. intra-specific diversification, is also regarded as a potential lever to decrease insect pest population. However, there is a lack of studies on the effects of combining these two levels of intra-field plant diversification on aphids and their generalist ground-dwelling predators. In this regard, we investigate the effects of the use of barley variety mixture and intercropping with clover on aphid population and ground-dwelling arthropods. Field trials were therefore set up on 9 organic commercial farms in South-East of France during the 2019-2020 and 2020-2021 growing seasons. In spring, aphids and ground-dwelling arthropods were monitored on different crop treatments corresponding to pure barley, mix of two barley varieties and barley associated or not with clover. Among the types of plant diversification tested, only inter-specific diversification showed effects on aphids and their ground-dwelling predators. For aphids, the effect of clover was different depending on the species considered. For ground-dwelling arthropods, the effect of clover was different depending on the taxon considered, and opposite effects were observed between the two years studied.

1. Introduction

Cereal aphids (Hemiptera: Aphididae) are major insect pests of grain cereals in Europe. In spring, the main cereal aphid species present in grain cereals are *Sitobion avenae* (Fabricius, 1775), *Metopolophium dirhodum* (Walker, 1849) and *Rhopalosiphum padi* (Linnaeus, 1758). They feed on phloem sap, reducing the transport of phloem to sink organs, particularly the grains (Poehling *et al.*, 2007). Yield losses are caused principally because of phloem sap loss but also through the deposition of honeydew, which leads to reduced photosynthesis by the development of sooty mould (Rossing, 1991). Aphid infestations in spring can also affect the grains baking quality by causing changes in protein composition (Oakley *et al.*, 1993). The severity of damages caused by cereal aphids in spring depends on several parameters (Poehling *et al.*, 2007). First, damages depend on the aphid species and its feeding site. For example, *R. padi* and *M. dirhodum* feed on cereal leaves, while *S. avenae* attacks ears as soon as they emerge. Cereal yield loss per aphid is doubled when ears are attacked compared to leaves (Niehoff and Stäblein, 1998). Second, cereal growth stages can be impacted differently. The flowering-stage is the most sensitive one to aphids,

the yield loss per aphid being doubled when infestation occurs at that time compared to later stages such as green ripeness (Wratten *et al.*, 1979; Entwistle and Dixon, 1987; Möwes *et al.*, 1997). Third, the level of aphid infestation determines the amount of damage caused. Few individuals per tiller generally result in undetectable yield losses, mainly because of plant tolerance (Poehling *et al.*, 2007). Entwistle and Dixon (1987) found a 1.4% to 4.4% yield loss for 10 aphids/tiller and a 5.7 to 15.6% yield loss for 40 aphids/tiller.

The biological control of herbivores is particularly important and often strong in agricultural systems (Halaj and Wise, 2001; Symondson *et al.*, 2002). The natural enemy community of cereal aphids is highly diverse and present different levels of specificity. There are generalist predators such as spiders (Araneae), ground beetles (Coleoptera: Carabidae) and rove beetles (Coleoptera: Staphylinidae), which are primarily ground-dwelling predators, and specialist predators such as ladybirds (Coleoptera: Coccinellidae), hoverflies (Diptera: Syrphidae) and lacewing (Neuroptera: Chrysopidae), whose adults fly and can lay their offspring within the aphid colonies. The most specialised aphid natural enemies are parasitoid wasps (Hymenoptera: Aphidiidae) (Wratten and Powell, 1991). The present study will focus on cereal aphids and their ground-dwelling generalist predators.

Several studies found that generalist predators can reduce cereal aphid populations (Edwards *et al.*, 1979; Chiverton, 1986; Helenius, 1990; Dennis and Wratten, 1991; Lang, 2003; Östman *et al.*, 2003; Schmidt *et al.*, 2003). Their effect on aphids is usually significant early in the season when aphids start to colonise fields (Chiverton, 1986; Marc *et al.*, 1999; Lang, 2003). Indeed, generalist predators can be abundant prior aphid outbreak because they do not rely exclusively on aphids and can feed on other prey within the field. Then, their effect on controlling aphids can continue until aphid populations peak (Winder, 1990; Schmidt *et al.*, 2003). Specialist predators are the most effective during high aphid densities later in the season (Chambers *et al.*, 1983; Freier *et al.*, 1997).

Some agroecological infrastructures can support and sometimes increase the populations of generalist predators in agroecosystems. For example, grass strips implemented around fields can provide an overwintering habitat and increase spring colonisation rate in the neighbouring field (Thomas *et al.*, 1992). However, some generalist predators do not have great dispersal abilities, such as many ground beetles and lycosid spiders which disperse by walking and only slowly colonise fields from adjacent habitats (Coombes and Sotherton, 1986; Duffield and Aebischer, 1994; Wallin, 1985). Thus, a strong edge effect can be observed with ground predator densities being higher near the field border and decreasing with distance into the field (Coombes and Sotherton, 1986; Bowie *et al.*, 1999). In large fields, increasingly common in agricultural landscapes, the edge:field area ratio is low and ground predators may stay close to the boundaries, allowing aphid populations to develop in the centre of cereal fields without any biological control (Coombes and Sotherton, 1986). Thus, other types of agroecological practices (Wezel *et al.*, 2014) could be designed and implemented to support the presence of generalist predator populations closer to the cereal aphid location, in order to decrease their outbreak.

The inter-specific plant diversification within a field, such as a cereal-legume association, can create microhabitats suitable for ground predators, as close as possible to the aphid populations and potentially regulate them via top-down mechanisms (Gurr *et al.*, 2004). In addition, this type of practice has been shown to provide many other services such as weed control, nitrogen supply

or soil erosion protection with plant cover after the cereal harvest (Bergkvist *et al.*, 2011; Amossé, 2013). This type of plant diversification can also act directly on aphids through bottom-up mechanisms (Gurr *et al.*, 2004), e.g. disturbing them in their search for host plants (Mansion-Vaquié *et al.*, 2020).

Another type of plant diversification within the field has shown promising effects on cereal aphids: intra-specific plant diversification through variety mixtures (Pettersson *et al.*, 1999; Ninkovic *et al.*, 2002; Shoffner and Tooker, 2013; Grettenberger and Tooker, 2016).

In the present study, field experiments were conducted to test the effect of intra-field plant diversification on cereal aphid populations, as well as on ground-dwelling arthropods. Two types of plant diversification (intra- and inter-specific) were tested separately as well as in combination in order to observe if synergistic effects exist. The following hypotheses were tested:

- 1) Inter-specific plant diversification reduces cereal aphid populations and increases ground-dwelling arthropod populations.
- 2) Intra-specific plant diversification reduces cereal aphid populations within the field.
- 3) The two types of plant diversification combined amplify the effects of plant diversification with decreased cereal aphid populations.

2. Materials and methods

2.1. Field experiment design

Field experiments were established on commercial farms under organic agriculture in the Rhône-Alpes region (South-East of France) over two consecutive winter barley growing seasons (2019-2020 and 2020-2021). Each field experiment was composed of six different treatments: three treatments with winter barley alone: barley variety Amistar, variety Margaux, and a mixture of the two varieties; and three treatments with winter barley associated with clover: barley variety Amistar/clover, variety Margaux/clover and a barley variety mixture/clover. Each treatment was seeded on a plot of 24 m x 50 m (Figure 5.1). The winter barley was sown at 150 kg/ha (355 seeds/m²), with a substitutive design for the barley mixture. The clover cover was composed with a mixture of three clover species in order to provide a good ground coverage during the barley growing season, by the complementarity of their development cycles. The clover mixture was sown at 13 kg/ha (5 kg/ha of *Trifolium alexandrinum*, 5 kg/ha of *Trifolium incarnatum* and 3 kg/ha of *Trifolium repens*). Winter barley and clover were sown simultaneously in the respective treatments, in October. The field experiments were conducted on five fields in 2019 and five others in 2020, but one field was excluded from the analysis due to poor clover development in spring 2021. Field experiments were not treated with pesticides and weeds were mechanically controlled (one harrowing pass between November and March) except in the clover growing areas. Fields were fertilised once in January/February with organic fertilisers.

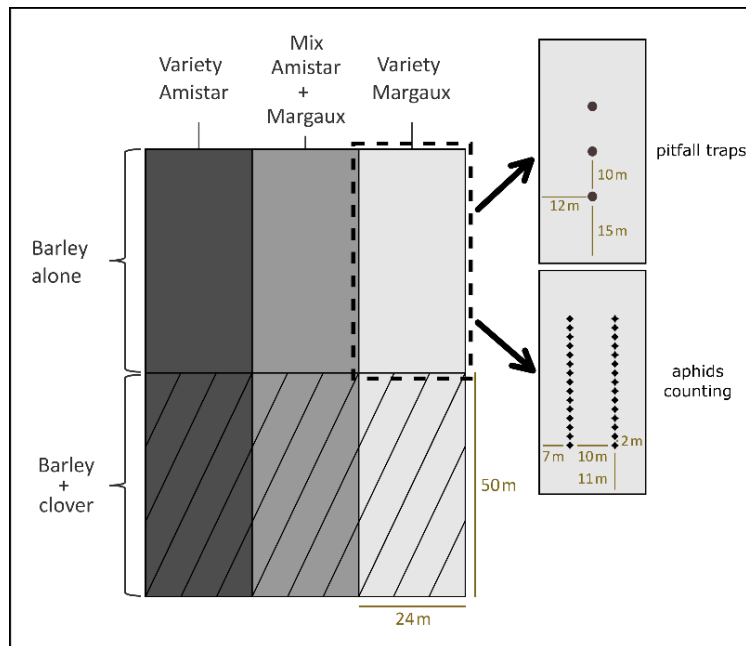


Figure 5.1. Design of the field experiment and protocols implemented in spring. Two factors are tested in six treatments: the "variety" factor (Amistar, Margaux and Mixture) and the "cover crop" factor (with or without clover).

2.2. Weather monitoring and weather conditions during the field experiment

In the field experiments, temperatures (maximum and minimum) were recorded every 30 minutes using a Tiny-tag data logger (Tinytag plus 2, Gemini Data Loggers, Chichester, UK). Data loggers were kept during the whole experiment period. The daily rainfall amounts were obtained from the Lyon-Bron weather station and are available on the Prévisionmétéo.ch website.

The climatic conditions during the two years of the field experiment were very contrasted, with a warm and rather dry spring in 2020 and a cool and rainy spring in 2021. Concerning rainfall, spring 2021 was around twice as rainy as spring 2020 (258 mm and 138 mm of rain in total over the period 01/03 - 01/06 respectively), notably due to heavy rainfall throughout the month of May 2021 (Figure 5.2). In 2020, the monitoring periods of ground-dwelling arthropods (see section 2.4) occurred during periods without rain while it was not the case in spring 2021 (sampling period 1 to 5 with respectively 10 mm, 0.5 mm, 11 mm, 6 mm, 5 mm of precipitation). Concerning temperatures, spring 2021 was colder than spring 2020 (10.8°C and 13.3°C on average over the period 01/03 - 01/06 respectively). Notable differences appeared especially in April (-4°C on average in 2021 compared to 2020) and in May (-3°C on average in 2021 compared to 2020) (Figure 5.2).

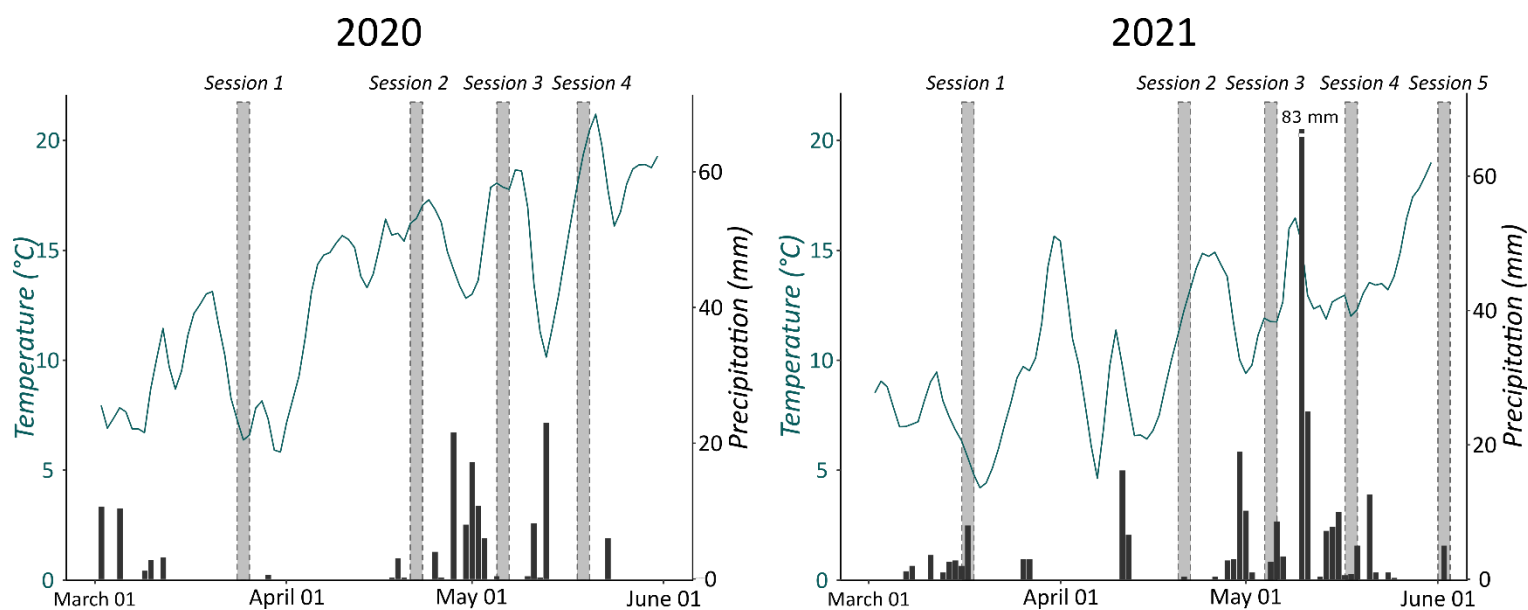


Figure 5.2. Temperature and precipitation recorded in spring. Mean daily temperatures recorded on the trials averaged over 3 days (—). Daily precipitation recorded on the reference climate station Lyon-Bron (■). 48-hours trapping periods with pitfall traps (■).

2.3. Monitoring of cereal aphids

Aphids were monitored on 30 barley tillers per treatment, along two parallel transects (Figure 5.1). Per transect, aphids were counted on 15 tillers, with a distance of 2 meters between two tillers. The transects were spaced 10 meters apart, and 7 meters from the plot borders to limit edge effects. Each sampled tiller was carefully cut and the number of aphids on it was recorded. For each aphid individual, species, development stage (nymph or adult), and, in case of adults, morph (alate or wingless) were recorded. This aphid monitoring was carried out at four stages of the cereal development in spring 2020 and 2021: booting (end-March), heading (end-April), flowering (early-May) and milk development (late-May), i.e. a period of 1 month between the first two sampling periods and two weeks between the next sampling periods.

2.4. Monitoring of ground-dwelling arthropods

Ground-dwelling arthropods were monitored using three pitfall traps placed in the central line of each treatment. Traps were placed 10 m from each other and 15 m away from the plot edges (Figure 5.1). Pitfall traps (\varnothing 10 cm) were half-filled with a 30% propylene glycol solution supplemented with a drop of odourless soap and covered by a plastic plate (\varnothing 17 cm) to protect the samples (rain, bycatch). The traps were opened for 48 hours. Ground beetles and spiders were identified at the species and at the family level, respectively, while rove beetles were counted without further identification. Four trapping periods were set up, at the same time as cereal aphid monitoring, from end-March to end-May. A supplementary sampling period was added at the beginning of June in 2021.

2.5. Monitoring of crop development

The development of the different crops was monitored using 0.25 m² quadrats, randomly replicated three times per treatment. In each quadrat, the percentage of barley and clover ground cover was recorded by visual estimation. The average height of each crop was measured (barley and the three clover species) and their growing stage was recorded. Four sampling periods of measurement were performed per crop cycle from end-March to end-May in 2020 and 2021.

2.6. Statistical analyses

Based on the aphid data collected on the field experiment, aphid occurrence (i.e. the probability of encountering at least 1 aphid per tiller) and aphid abundance (i.e. the number of aphids per tiller) were calculated for cereals aphids (all species grouped together) and individually for *M. dirhodum*, *S. avenae* and *R. padi*.

Concerning ground-dwelling arthropods, activity-density (i.e. number of individuals per pitfall trap) was calculated. For ground beetles, activity-density was calculated for all species grouped together and for the two dominant species individually (*Anchomenus dorsalis* and *Poecilus cupreus*). For spiders, activity-density was computed for all families grouped together and for the two dominant families separately (Lycosidae and Linyphiidae). A species or a family was considered “dominant” when it was present with $\geq 9\%$ of total abundance per year. Concerning rove beetles, activity-density was calculated for all individuals grouped together.

These variables were analysed using Generalized Linear Mixed Effects Models (GLMM). The factor *variety* (3-levels factor: Amistar, Margaux and Mixture), the factor *clover* (2-levels factor: with clover and without clover), the factor *year* (2-levels factor: 2020 and 2021), and their interactions were used as fixed effects. A random effect of the site and of the sampling period were added to account for variations of aphid populations between sites and between sampling periods.

$$\text{Variable tested} \sim \text{variety} + \text{clover} + \text{year} + \text{variety} \times \text{clover} + \text{variety} \times \text{year} + \text{clover} \times \text{year} + (1 | \text{sampling period}) + (1 | \text{site})$$

A binomial family link was used for occurrence variables and a negative binomial link for abundance and activity-density to account for overdispersion. The significance of fixed factors and their interaction was determined by a likelihood ratio test (LRT). Statistical analyses were performed using R version 4.1.1 with lme4 package for GLMM. drop1 function was used to perform LRT.

3. Results

3.1. Crop development during the spring period

Crop development (clovers and barley) was fairly consistent over the two years. Regarding the clover ground cover, there was no significant difference between the two years. The only difference occurred at the end-March sampling period, when the clover covered 10% more ground in 2021 than in 2020 (Figure 5.3a). Barley ground cover was higher throughout the season in 2021 compared to 2020, covering on average 20% more ground. Concerning the height of the different crops, they were similar between the two years. The only notable difference occurred at the end-April sampling period, when the crops were higher in 2020 than in 2021, except for the white clover (10 cm, 19 cm and 8 cm higher respectively for barley, *T. incarnatum*, *T. alexandrinum*) (Figure 5.3b). During the spring monitoring, the two clovers *T. alexandrinum* and *T. repens* did not come into flower. *T. incarnatum* clover was in flower at the end-April sampling period in 2020 and at the early-June sampling period in 2021 (Figure 5.3b), reflecting a later crop development the second year, probably related to the climatic conditions.

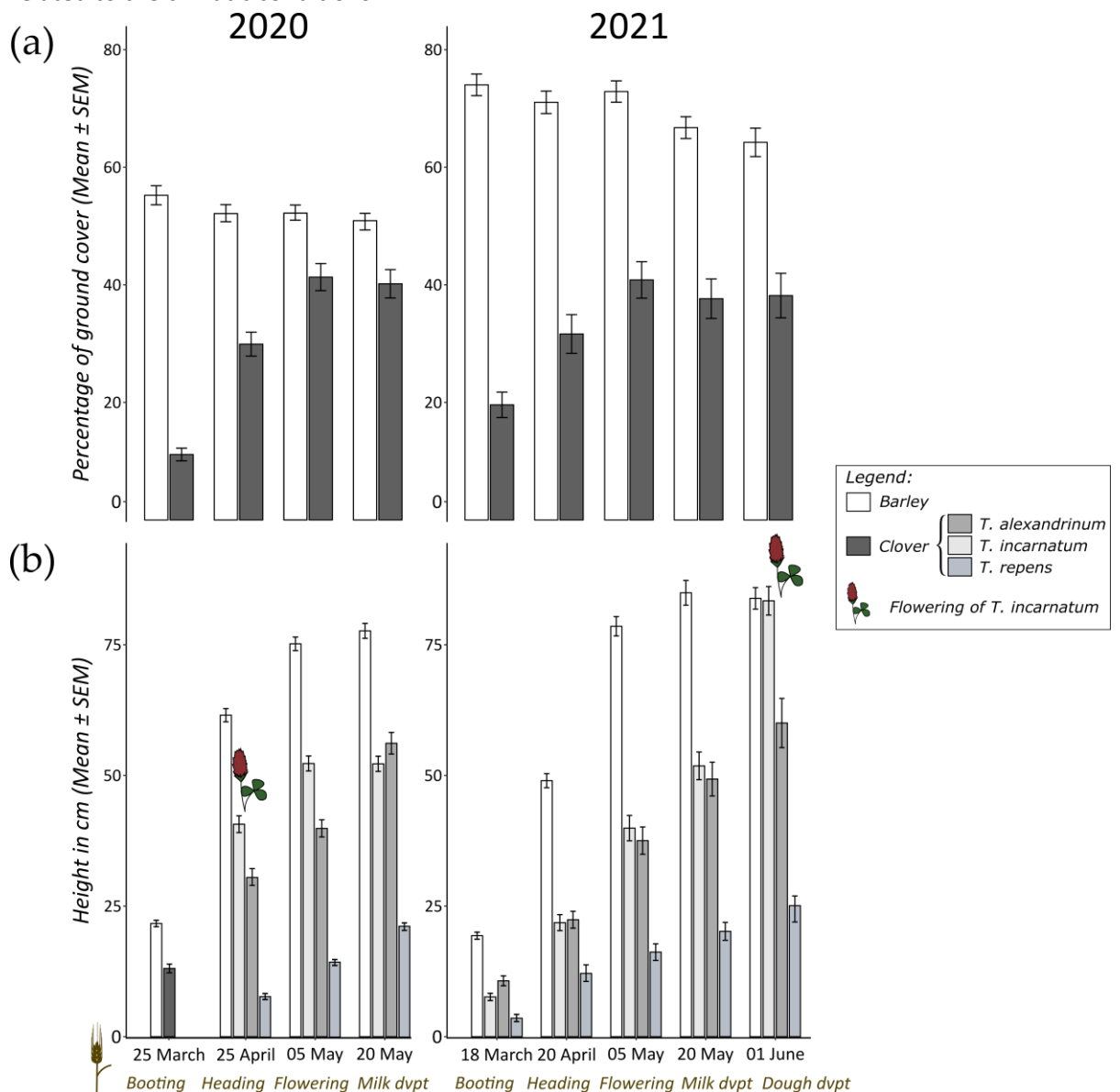


Figure 5.3. Development of barley and clover cover in spring. (a) Ground cover and (b) Height of barley and clovers (Mean ± SEM) during the monitoring period in 2020 (n=90 and n=45 for treatment without and with clover respectively) and 2021 (n=72 and n=36 for treatment without and with clover respectively).

3.2. Cereal aphids

3.2.1. Cereal aphid species and abundance

During the 2 months monitoring (end-March to end-May), a much higher number of aphids were found in one of the years. In 2020, 1617 aphids were counted in total on the field experiments, whereas there were 8728 aphids counted in 2021. Thus, a significant inter-year difference in aphid numbers could be noticed, being almost 7 times higher in 2021 than in 2020 (Table 5.1). *M. dirhodum* and *S. avenae* were the two dominant aphid species present (both together accounting for 86% of total counts in 2020 and 77% in 2021). *R. padi* was the third most represented species in a substantially lower proportion, and with a varying abundance between the two years of monitoring (Table 5.1).

Table 5.1. Aphid species observed on barley tillers in spring in the two years of monitoring.

Species	Year *		Total *
	2020	2021	
<i>Metopolophium dirhodum</i>	730 (45%)	3394 (39%)	4124 (40%)
<i>Sitobion avenae</i>	664 (41%)	3372 (39%)	4036 (39%)
<i>Rhopalosiphum padi</i>	75 (5%)	1429 (16%)	1504 (14%)
<i>Schizaphis graminum</i>	0 (0%)	391 (4%)	391 (4%)
<i>Rhopalosiphum maidis</i>	87 (5%)	53 (1%)	140 (1%)
<i>Other cereal aphids sp.</i>	61 (4%)	89 (1%)	150 (1%)
Total	1617	8728	10345

* Total number of aphids per species and relative proportion (in brackets) per each year of monitoring and for both years combined. n=3600 tillers observed in 2020 and n=2880 tillers observed in 2021.

3.2.2. Cereal aphid temporal dynamic

Per monitoring year, aphid occurrence and abundance showed similar temporal dynamic patterns (Figure 5.4a, 5.4b). Each year, the temporal dynamics of the aphids showed a single peak curve, which occurred at the heading stage of barley (on average, 27% of the tillers colonised and 0.8 aphid/tiller in 2020 and 62% of the tillers colonised and 4 aphids/tiller in 2021). Then, the populations decreased at the flowering stage of barley, the most sensitive stage to aphids in spring. In each monitoring year, the occurrence curve of *S. avenae* was above or similar to the one of *M. dirhodum* and this observation was reversed for the abundance curves. Thus, *M. dirhodum* infested fewer tillers than *S. avenae*, but presented a larger population per infested tiller. During the last monitoring of 2020, at the milk development stage of barley, it can be noted that *S. avenae* population declined more slowly than the other species. In 2021 *R. padi* population dropped faster than the others.

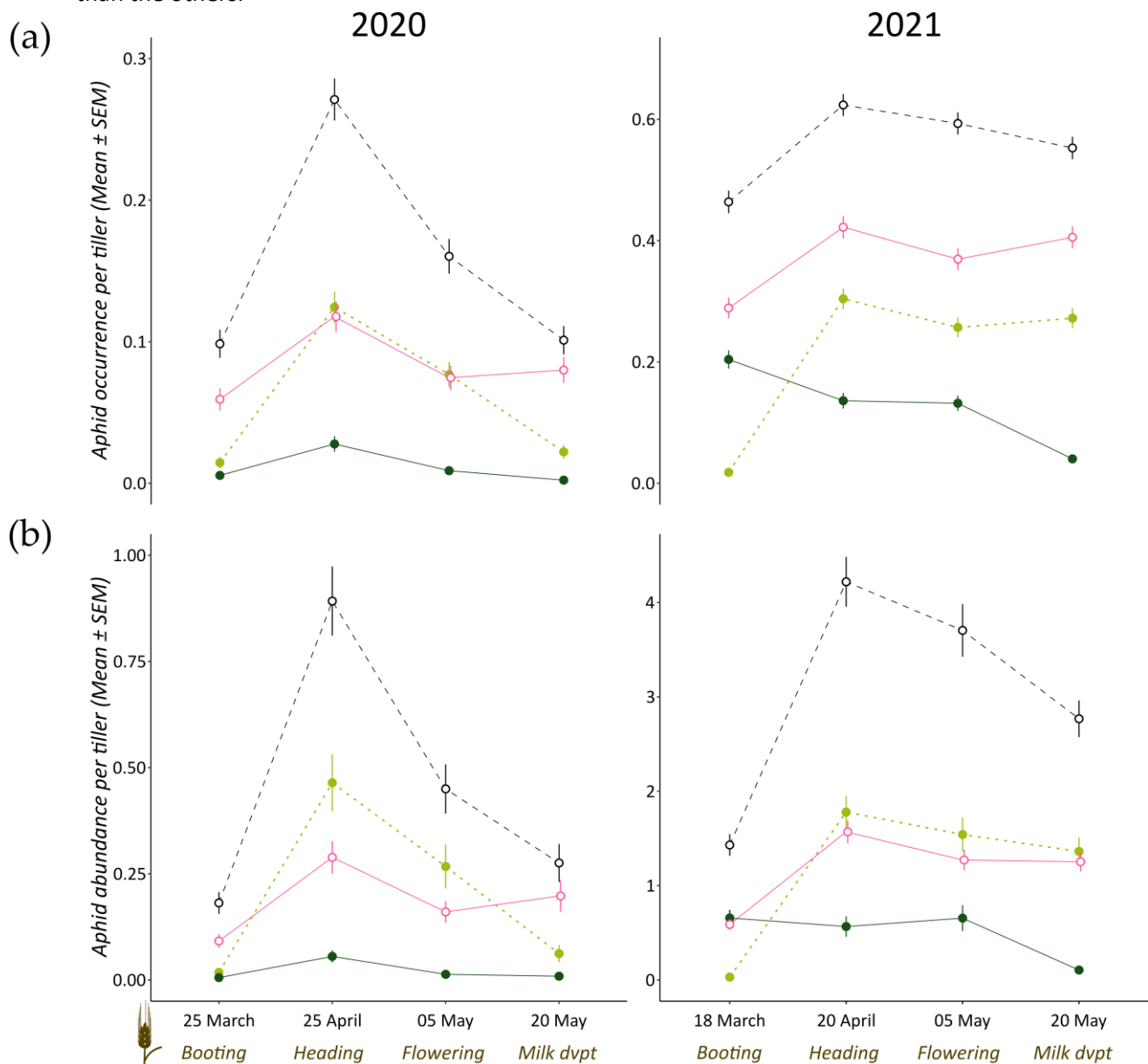


Figure 5.4. Monitoring of aphid populations on barley tillers in spring. (a) Occurrence and **(b)** Abundance (Mean \pm SEM) of: All cereal aphids species ($- \circ -$), *Metopolophium dirhodum* ($- \bullet - \bullet -$), *Sitobion avenae* ($- \circ -$), *Rhopalosiphum padi* ($- \bullet -$). Per sampling period: n=900 tillers observed in 2020 and n=720 in 2021. The development stages of barley are indicated below the x-axis (“milk dvpt” means milk development-stage).

3.2.3. Effects of plant diversification on cereal aphids

The different explanatory variables showed higher significant effects on the abundance of cereal aphids, rather than on their occurrence. The only explanatory variable that was consistently significant for cereal aphid abundance and occurrence was the *year* ($p \leq 0.04$, Table 5.2), which is related to the higher aphid populations in spring 2021 compared to 2020.

3.2.3.1. Effects on aphid abundance

Intra-specific diversification did not decrease aphid abundance compared to each variety alone. Indeed, the abundance in the mixture was: either equivalent to the abundances of the two varieties (case of all species combined, *M. dirhodum* and *R. padi* in 2021); either equivalent to one variety and higher to the other (case of *R. padi* in 2020 and *S. avenae* in 2021 where abundance in the mixture was higher than in Margaux and Amistar respectively); or equivalent to one variety and lower to the other (case of *S. avenae* in 2020 where the abundance in the mixture was lower than in Margaux). In any case the abundance in the mixture was both lower than the abundance in Margaux and the abundance in Amistar.

Inter-specific diversification had no effect on the abundance of all aphid species combined (for the *clover* factor, $p=0.41$, Table 5.2), but had significant effects for each of the three individual aphid species ($p < 0.001$ for *M. dirhodum* and *R. padi* and $p=0.02$ for *S. avenae*). For *M. dirhodum*, only the *clover* factor was significant, the abundance of this species was decreased in the presence of clover (20% decrease) (Figure 5.5b). For *S. avenae*, the interactions *clover* \times *variety* and *clover* \times *year* were also significant ($p < 0.001$). Graphically, it can be observed that in 2020, *S. avenae* abundance was increased in Amistar/clover and Margaux/clover treatments compared to Amistar and Margaux alone, and in 2021, it was increased in Margaux/clover and decreased in mixture/clover treatments (Figure 5.5c). For *R. padi* species, its abundance was decreased in the presence of clover, in the mixture in 2020 and in Margaux in 2021 (Figure 5.5d).

Intra and inter-specific plant diversification combined did not decrease aphid abundance to a higher degree than each type of diversification alone. Indeed, in any case, the abundance in the mixture/clover treatment was lower than in the mixture alone and also lower than in Amistar/clover and Margaux/clover treatments (Figure 5.5). For *R. padi* in 2020 and *S. avenae* in 2021 their abundance was decreased in the mixture/clover treatment compared to the mixture alone. But the mixture/clover was equivalent to the Margaux/clover and to the Amistar/clover treatments for *R. padi* and *S. avenae* respectively (Figure 5.5c and 5.5d). Thus the abundance in the mixture/clover was not lower than in all other treatments.

Table 5.2. Statistical test results on the effect of different factors and their interactions on cereal aphid populations in spring.

Variable tested		p-value *					R ² *		
		V	C	Y	V × C	V × Y	C × Y	R ² m	R ² c
Aphid abundance	All cereal aphid species	0.005	0.41	< 0.001	0.06	0.11	0.23	0.25	0.39
	<i>M. dirhodum</i>	0.70	< 0.001	0.01	0.58	0.47	0.86	0.11	0.62
	<i>S. avenae</i>	< 0.001	0.02	< 0.001	< 0.001	< 0.001	< 0.001	0.21	0.32
	<i>R. padi</i>	< 0.001	< 0.001	0.04	0.02	< 0.001	0.40	0.09	0.29
Aphid occurrence	All cereal aphid species	0.44	0.33	< 0.001	0.11	0.04	0.67	0.18	0.25
	<i>M. dirhodum</i>	0.75	0.03	0.004	0.73	0.12	0.26	0.05	0.21
	<i>S. avenae</i>	0.12	0.08	< 0.001	0.03	0.03	0.008	0.13	0.18
	<i>R. padi</i>	0.65	0.77	0.02	0.59	0.60	0.64	0.06	0.15

V: variety; C: clover; Y: year; V × C: interaction between variety and clover; V × Y: interaction between variety and year; C × Y: interaction between clover and year. * p-values from GLMM models. P-values < 0.05 are written in bold. ** R²m: marginal R-squared value considering only the variance explained by the fixed effects; R²c: conditional R-squared value considering the variance explained by both fixed and random effects.

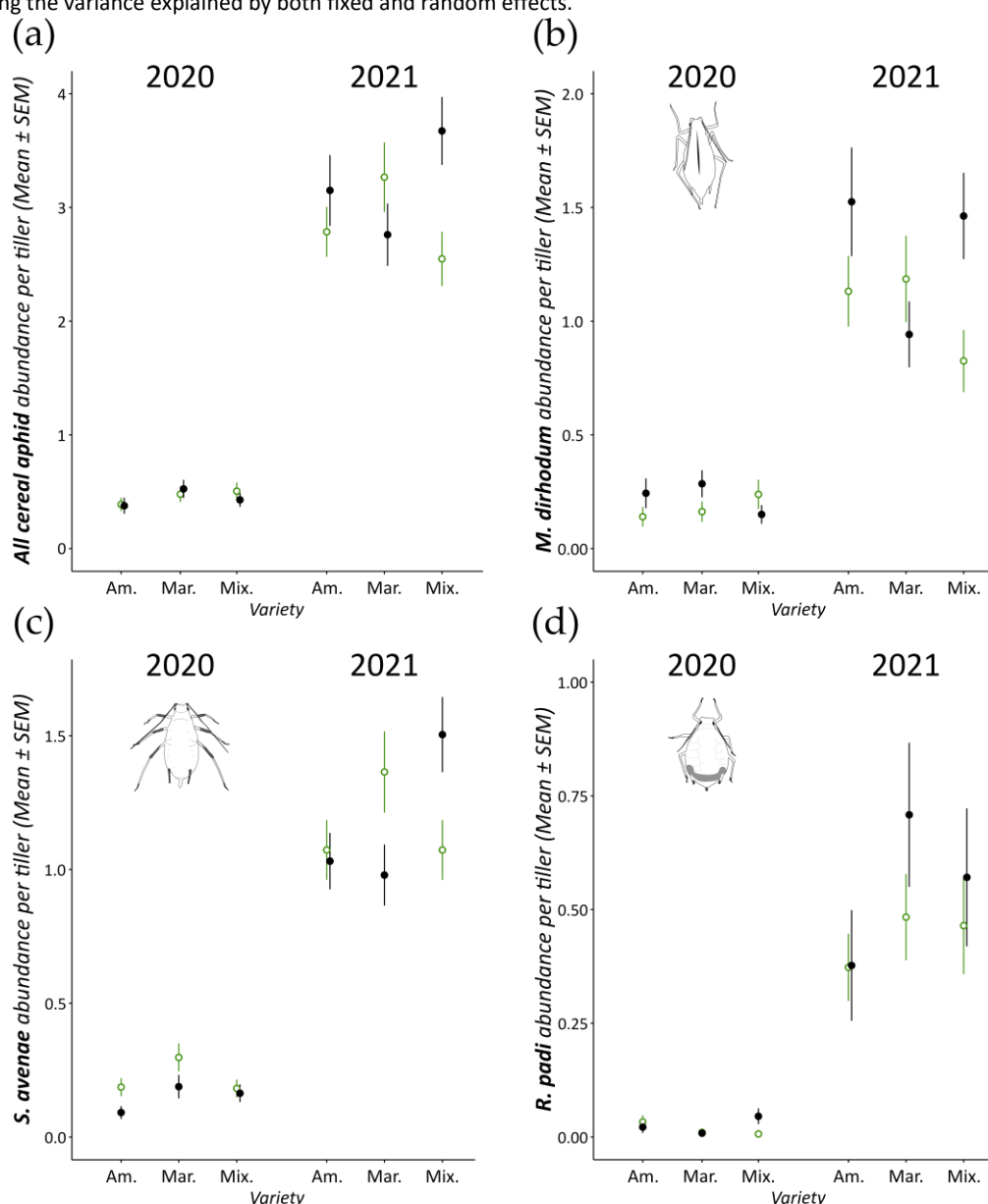


Figure 5.5. Abundance of cereal aphids in spring regarding variety mixture or sole variety and barley intercropped or not with clover. Mean ± SEM per tiller of: (a) All cereal aphid species, (b) *M. dirhodum*, (c) *S. avenae*, (d) *R. padi*. Per treatment: n=600 tillers observed in 2020 and n=480 in 2021. Treatment with clover (○); Treatment without clover (●).

3.2.3.2. Effects on aphid occurrence

Intra-specific diversification did not decrease the occurrence of cereal aphids compared to varieties alone. For *R. padi* and *M. dirhodum*, the occurrence between the Amistar, Margaux and mixture treatments were equivalent. For all species combined and *S. avenae*, the occurrence in the mixture was equivalent to one variety and higher than the other one (All species combined where the mixture was higher than Amistar in 2020 and than Margaux in 2021; and *S. avenae* in 2020 where the mixture was higher than Amistar). In 2021 for *S. avenae*, the occurrence in the mixture was even higher than the occurrence in Amistar and in Margaux (significant effect of intra-specific diversification, but in the opposite direction of the expected one) (Annexe 5c).

Inter-specific diversification had still no effect on all aphid species combined. The factor *clover* had again a significant effect on *M. dirhodum*, decreasing its occurrence ($p=0.03$ Table 5.2 and 12% reduction). In contrast to its abundance, *clover* had no effect on the occurrence of *R. padi* (Annexe 5d). Concerning *S. avenae*, the same results as for the abundance were observed: in 2020, the occurrence of *S. avenae* was increased in the Amistar/*clover* and Margaux/*clover* treatments compared to Amistar and Margaux alone, and in 2021, it was increased in the Margaux/*clover* treatments and decreased in the mixture/*clover* treatments (Annexe 5c).

The two types of diversification combined, as for abundance, did not decrease aphid occurrence to a higher degree than each type of diversification alone (Annexe 5).

3.3. Ground-dwelling arthropods

3.3.1. Ground-dwelling arthropods abundance and temporal dynamic

Over the two years studied, the most represented taxon in pitfall trap catches was the spiders (2087 individuals in total), followed by ground beetles (1882 individuals) and rove beetles (963 individuals) (Table 5.3). However, for each taxon, inter-annual abundance variations were observed: 1.6 times more ground beetles and 3.5 times more spiders were caught in 2020 than in 2021. Conversely, 1.2 times more rove beetles were caught in 2021 than in 2020.

Table 5.3. Ground-dwelling arthropods collected in pitfall traps in spring in the two years of monitoring.

Lower level taxon		Year		Total *
		2020	2021	
Rove beetles	Total	441	522	963
Ground beetles	<i>Anchomenus dorsalis</i>	503	230	733 (39%)
	<i>Nebria salina</i>	24	158	182 (10%)
	<i>Poecilus cupreus</i>	110	70	180 (10%)
	<i>Asaphidion stierlini</i>	98	13	111 (6%)
	<i>Trechus quadristriatus</i>	62	22	84 (4%)
	<i>Metallina properans</i>	65	15	80 (4%)
	<i>Pseudoophonus rufipes</i>	34	29	63 (3%)
	<i>Harpalus distinguendus</i>	17	36	53 (3%)
	<i>Microlestes minutulus</i>	31	7	38 (2%)
	<i>Harpalus affinis</i>	5	29	34 (2%)
	<i>Demetrias atricapillus</i>	11	22	33 (2%)
	<i>Brachynidius sclopeta</i>	27	5	32 (2%)
	<i>Amara aenea</i>	15	12	27 (1%)
	<i>Amara similata</i>	19	6	25 (1%)
	<i>Harpalus dimidiatus</i>	17	3	20 (1%)
	Other (39 species)	136	51	187 (10%)
	Total	1174	708	1882 (100%)
Spiders	Lycosidae	877	173	1050 (50%)
	Linyphiidae	339	185	524 (25%)
	Thomisidae	194	28	222 (11%)
	Gnaphosidae	147	33	180 (9%)
	Pachygnatha	30	5	35 (2%)
	Theridiidae	11	9	20 (1%)
	Other (13 families)	34	22	56 (2%)
	Total	1632	455	2087 (100%)
Other Arachnids	Harvestmen	61	1	62
	Red mites	21	39	60

* Total number of ground-dwelling arthropods per taxon. Relative proportion of the dominant ground beetle species and spider families (i.e. representing at least 1% of the total) counted during the whole monitoring period. n=360 pitfall traps in 2019 and n=360 pitfall traps in 2020.

In 2020, the activity-density temporal curve of ground beetles and rove beetles showed a single peak, occurring at the flowering stage of barley, in late May. The spider activity-density curve showed a constant progression over the spring and a maximum at the last sampling period. In 2021, the populations were relatively low and stable from end-April to end-May. It was followed by an increase in catches in the last sampling period of early-June (Figure 5.6).

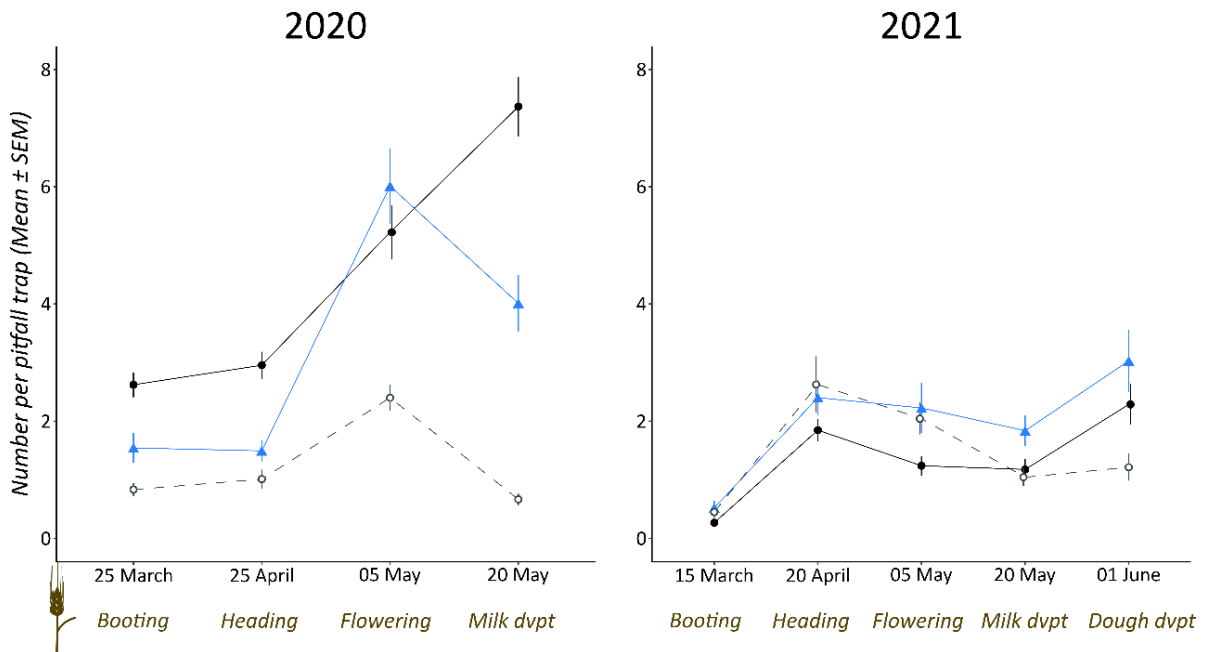


Figure 5.6. Ground-dwelling arthropod activity-density in spring. Mean \pm SEM per pitfall trap of: Ground beetles ($\text{---}\blacktriangle\text{---}$), Spiders ($\text{---}\bullet\text{---}$), Rove beetles ($\text{---}\circ\text{---}$). Per sampling period: $n=90$ pitfall traps in 2020 and $n=72$ in 2021. The development stages of barley are indicated below the x-axis (“milk dvpt” means milk development-stage).

3.3.2. Taxonomic diversity of ground-dwelling arthropods

The 1882 ground beetles caught during the two years of monitoring belonged to 56 different species. The three main abundant species were *Anchomenus dorsalis* (*A. dorsalis*), *Poecilus cupreus* (*P. cupreus*), and *Nebria salina* (*N. salina*), which together represented 59% of all individuals captured (Table 5.3). The 2087 spiders captured belonged to 19 different families. The two main families were lycosids (Lycosidae) and linyphids (Linyphiidae), which together represented 75% of total captures.

3.3.3. Effects of plant diversification on ground-dwelling arthropods

The year factor was highly significant for the different ground-dwelling arthropods ($p < 0.001$, Table 5.4), except for rove beetles and linyphiids. For these different taxa, their activity-density was clearly higher in 2020 than in 2021 (Figure 5.7).

Intra-specific diversification and the two types of diversification combined had no effect on the activity-density of ground-dwelling arthropods. The factor *variety* and the *variety* \times *clover* interaction were not significant for any of the variables tested (Table 5.4).

Inter-specific diversification had significant effects on some ground-dwelling arthropod taxa. For rove beetles, the factor *clover* was significant ($p = 0.04$), the presence of clover slightly increasing their overall activity-density (increase of 23%). For ground beetles, the *clover* \times *year* interaction was significant for all species combined, as well as for the major species *A. dorsalis* and *P. cupreus* ($p < 0.001$). Indeed, we observe that in the presence of clover their activity-density was increased in 2020 (increase of 50%, 103% and 167% respectively) and decreased in 2021 (decrease of 35%, 33% and 51% respectively) (Figure 5.7d, 5.7e and 5.7f). For spiders, the *clover* \times *year* interaction was only significant for the Lycosidae family ($p < 0.001$). As for ground beetles, their activity-density increased in the presence of clover in 2020 (increase of 44%) and decreased in 2021 (decrease of 25%) (Figure 5.7b). Clover had no effect on the activity-density of the Linyphiidae family (Figure 5.7c).

Table 5.4. Statistical test results on the effect of different factors and their interactions on ground-dwelling arthropod populations in spring.

Variable tested		p-value *						R ² **		
		V	C	Y	V \times C	V \times Y	C \times Y	R ² m	R ² c	
Activity-density	Spiders	All families	0.06	0.27	< 0.001	0.84	0.72	0.06	0.33	0.54
		Lycosidae	0.57	0.08	< 0.001	0.71	0.75	0.001	0.29	0.39
		Linyphiidae	0.54	0.57	0.09	0.53	0.15	0.21	0.07	0.50
	Ground beetles	All species	0.79	0.28	< 0.001	0.72	0.44	< 0.001	0.04	0.60
		<i>Anchomenus dorsalis</i>	0.14	< 0.001	< 0.001	0.58	0.33	< 0.001	0.37	0.85
		<i>Poecilus cupreus</i>	0.30	0.05	< 0.001	0.20	0.94	< 0.001	0.26	0.71
	Rove beetles	0.27	0.04	0.71	0.83	0.73	0.79	0.01	0.31	

V: variety; C: clover; Y: year; V \times C: interaction between variety and clover; V \times Y: interaction between variety and year; C \times Y: interaction between clover and year.

* p-values from GLMM models. P-values < 0.05 are written in bold.

** R²m: marginal R-squared value considering only the variance explained by the fixed effects; R²c: conditional R-squared value considering the variance explained by both fixed and random effects.

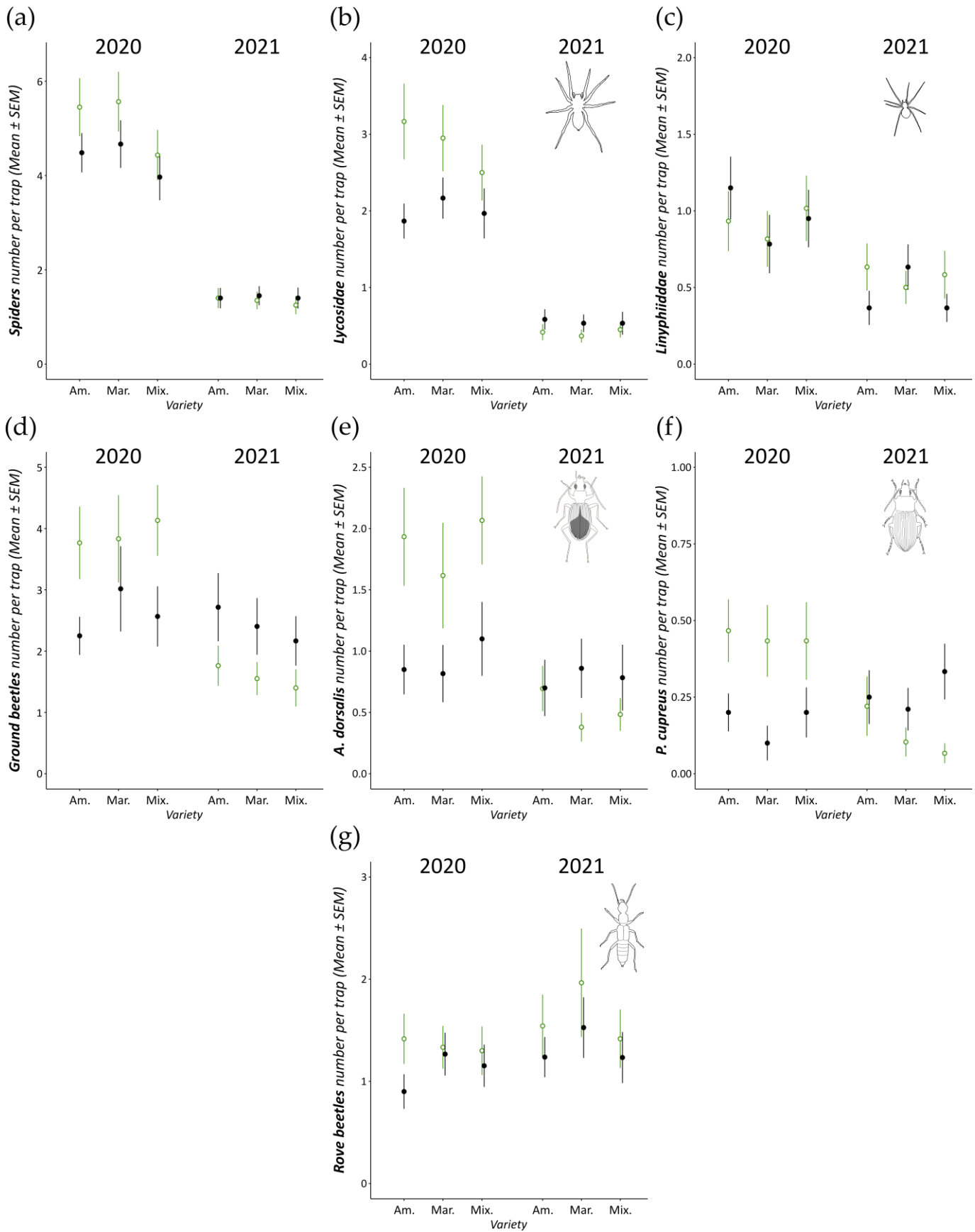


Figure 5.7. Activity-density of ground-dwelling arthropods in spring regarding variety mixture or sole variety, and barley intercropped or not with clover. Mean ± SEM per pitfall trap of: (a) All spider families, (b) Lycosidae family, (c) Linyphiidae family, (d) All ground beetle species, (e) *A. dorsalis*, (f) *P. cupreus* (g), Rove beetles. Per treatment: n=60 pitfall traps in 2020 and n=60 in 2021. Treatment with clover (○); Treatment without clover (●).

4. Discussion

In the present study several types of intra-field plant diversification were tested, with intercropping and crop variety mixture, in order to investigate their potential to decrease cereal aphid populations and increase the presence of their generalist ground-dwelling predators.

4.1. Intra-specific plant diversification reveals no significant effect on aphid populations

Intra-specific diversification did not lead to a decrease in cereal aphid populations, regardless of the species considered. In most cases, the populations in the mixture were equivalent to those in one of the two varieties grown in pure and higher than the populations in the second variety, which is not of major interest in the context of biological regulation of aphids. In a similar field experiment testing intra-specific diversification in spring, Mansion-Vaquié *et al.* (2019) did not observe lower aphid populations in the wheat mixture compared to each variety alone, which is consistent with our results.

4.2. Inter-specific plant diversification reveals significant effects on aphid populations

Only inter-specific plant diversification revealed significant effects on cereal aphid populations, differing according to the species considered. For *M. dirhodum* the effect of clover was more pronounced than for the two other species (decrease in aphid abundance by 20% and occurrence by 12%). For *R. padi*, a tendency to a decrease of its abundance in presence of clover was detected. In contrast, a tendency to an increase in *S. avenae* abundance in the presence of clover was found. These tendencies should be confirmed or not by additional field experiments. These effects of inter-specific plant diversification on aphids could be explained by two types of mechanisms: direct or indirect effects of the non-host plant on pest (i.e. by disturbing the pest to find its host plant and by attracting its natural enemies, respectively). Within each mechanism, clover would therefore have opposite effects depending on the species considered.

Changes in aphid abundance could be explained by direct effects of the clover plant on aphids. First of all, depending on the aphid species, aphids could use different inter-plant movement pathways, explaining the varying effects of the clover cover. Indeed, the three dominant cereal aphid species appear on different parts of the plant (Dean, 1974; Dedryver and Robert, 1977). *S. avenae* is mostly found on the highest position, on the upper leaves, and rapidly colonises the ears as they emerge (Watt, 1979). *M. dirhodum* shows preference for an intermediate position, on the flag and lower leaves (Leather and Dixon, 1981), and *R. padi* is located lower on the plant, on stem or basal leaves (Leather and Dixon, 1981). When aphids move from one plant to another, it can be hypothesised that species occupying a high position on the host plant would use the leaf pathway to move from one plant to another (i.e. via the touching leaf tips) (Power, 1991), and that species with a low position would use the ground pathway (i.e. walking on the soil surface), in order to move at the lowest energy cost. The different clover species used in the field experiment have different heights and architectures. The taller *T. incarnatum* and *T. alexandrinum* could increase the number of connections between the cereal plants throughout the growing season and thus create additional "leaf bridges" favouring the movements of *S. avenae* which could finally increase its populations. The clovers, could on the opposite represent a barrier for the two other aphid species occupying a low position on the cereal. It could hinder their movements, decrease their probability of encountering the host plant and the time spent exploring wrong plant material. It consequently may increase their energy spending, decrease their global fitness and at the end their populations through bottom-up mechanisms (Gurr *et al.*, 2004).

Changes in aphid abundance could also be explained by indirect effects of the clover on aphids, via the natural enemies. The high biomass of the clover canopy could represent a "screen" between the barley foliage layer and the soil layer and protect some species from their natural enemies. Daily, cereal aphid colonies experience a high turnover (Fievet *et al.*, 2007), individuals moving voluntarily (Holmes, 1988) or falling to the ground (Kerzicnik *et al.*, 2010; Sopp *et al.*, 1987) after being dislodged by various disturbances such rain, wind or foliar enemies (Mann *et al.*, 1995; Dhalival and Singh, 1975; Holmes, 1983). Daily fall-off rates of cereal aphids can range in average from 20 to 35 % (Sunderland *et al.*, 1986; Sopp *et al.*, 1987; Winder, 1990; Winder *et al.*, 1994; Kerzicnik *et al.*, 2010) but may vary among aphid species. For example, *M. dirhodum* is known to fall off from its host plant more easily than *S. avenae* (Dewar *et al.*, 1982; Sunderland *et al.*, 1986). Once on the ground, if they are not consumed by ground-active predators or web-building spiders (Sunderland *et al.*, 1986; Winder, 1990; Harwood *et al.*, 2004), aphids can quickly return to their host plant (Sopp *et al.*, 1987). Thus, aphids in a high position, such as *S. avenae*, could benefit from the screen effect of the clover cover, that may decrease their risk of falling directly on the ground and thus of being exposed to foraging ground predators. Aphids in a low position (*M. dirhodum* and *R. padi*) would less or not benefit from this "protective screen" effect of the clover, and would be more exposed and easily consumed by ground predators (Winder *et al.*, 2013) through top-down mechanisms (Gurr *et al.*, 2004). In addition, some ground-beetle species, as *P. cupreus*, are able to climb on plants to prey upon aphids (Chiverton, 1988). Aphids with a low position would therefore have a higher probability of being consumed.

4.3. Importance of aphids as pest and economic impact

Although the clover showed different effects, or tendencies, depending on the cereal aphid species, it is important to consider that they do not have the same importance as pest, and have different economic impact. In spring, the species that can be most damaging on grain cereals is *S. avenae*. The intervention threshold is reached when there are three to five aphids per ear or 50% of ears colonised at the barley flowering stage or at the beginning of milk stage. Yield losses can reach up to 2.5 t/ha (Turpeau *et al.*, 2011). Thus, this tendency of the clover to increase *S. avenae* abundance need to be confirmed and the underlying mechanisms further investigated. For their part, *M. dirhodum* and *R. padi* are not particularly problematic species for cereals in spring, as they feed on leaves only (Niehoff and Stäblein, 1998) but can have a significant impact in autumn on young winter cereals as vectors of diseases. Therefore, it is also interesting to further explore the potential of intercropping practice to decrease their populations in spring and therefore contribute to the decrease of their overall populations in the landscape.

4.4. Potential regulation of aphids by ground-dwelling arthropods

Concerning ground-dwelling arthropods activity-density, only inter-specific plant diversification showed significant effects on them, with contrasted effects depending on the taxon and the year considered. In 2020 and in the presence of clover, ground beetle populations, all species pooled as well as the dominant species *A. dorsalis* and *P. cupreus* were increased. Interestingly, the two latter species are known to consume aphid (Sunderland and Vickerman, 1980; Chiverton, 1987; Staudacher *et al.*, 2018). Rove beetle populations were slightly increased in the presence of clover. The lycosids, composed of ground-active spiders, experienced an increase in their activity-density, which was not the case for the linyphids, represented by small spiders producing non-sticky horizontal webs close to the ground (Bristowe, 1971; Ford, 1977). However, the study of Gravesen (2008) found a positive effect of a clover cover on the linyphids. In the

present study, intraguild predation may have occurred, as it can be especially significant among ground arthropods, due to the trend of prey and predators to aggregate (Snyder and Wise, 1999; Winder *et al.*, 2001; Holland *et al.*, 2004). Linyphiid spiders may have been consumed in the clover treatments by larger arthropods, themselves enhanced by the clover canopy. Linyphiids may also have been hindered by the very dense clover canopy with its complex leaf architecture, impeding their ability to set up their webs or to move by ballooning (Greenstone *et al.*, 1987).

Despite the positive effect of clover on almost all taxa in 2020, it had a completely opposite effect in 2021, decreasing ground beetles and lycosids activity-density. These contrasting results on ground-dwelling arthropods depending on the year cannot be explained by a difference in the clover cover development, which were relatively similar over the two years. However, the climatic conditions recorded during the two years of monitoring were very different and could explain these antagonistic effects of the clover cover. In the spring 2020, the climate was warm and dry, a cereal monoculture, that had a significant proportion of bare soil highly exposed to sun can thus represents an unfavourable environment for ground predators (Ward *et al.*, 2011). In these conditions, the clover cover may have offered a more favourable microclimate for ground-dwelling arthropods (Lys *et al.*, 1994; Holland and Luff, 2000), decreasing temperatures and increasing humidity at ground level. The clover may also have provided additional food resources for generalist predators (Whitcomb and Godfrey, 1991), enhancing predator reproduction, survival and their activity-density (Whitcomb and Godfrey, 1991). In contrast, in 2021, the climate was colder and rainier, the clover canopy could have increased the humidity and delayed soil warming. Thus, a more open habitat such as the cereal crop could have been more favourable for soil arthropods than the clover cover. Finally, it should be kept in mind that when pitfall traps are set up in different habitats (e.g. cereals alone and cereals associated with a cover crop), the activity-density is biased to estimate relative abundance between the two habitat, as a denser vegetation cover can impair ground beetle movement and therefore reduce catches in pitfall traps (Heydemann, 1956). In the present study, activity-density in the treatments with clover may therefore have been underestimated.

Acknowledgements: We thank Garreth Kratz, Aurore Arnoult, Adeline Cadiergues for their technical assistance in the field monitoring, as well as Aurore Arnoult for her assistance in sorting the pitfall traps. We are grateful to the farmers that permitted to carry out this on-farm research.

Funding: This work was supported by the European Union's Horizon 2020 Research and Innovation programme, under Grant Agreement no. 773554 (EcoStack).

Part 2. Spring effect of intra-field plant diversification on aphid foliar enemies in winter barley crop

In the spring period, aphid foliar enemies were also monitored in the field experiment. The monitoring methods, the results and a discussion are presented in this supplementary data part.

1. Materials and methods

1.1. Aphid foliar enemies monitoring

Foliar enemies of aphids were counted during the aphid monitoring, on the same 30 tillers per treatment (see chapter 5 part 1). For the foliar predators, their development stage was recorded (egg, larva, pupa or adult). For the parasitoids, mummy abundance (i.e. last parasitoid larval instar) was monitored, as they are easily countable in the field. For each mummy, the emergence status of the parasitoid was monitored through the observation of emergence hole. Parasitism rate was calculated as the ratio of mummies to total aphids (including mummies).

This foliar enemies monitoring, as aphid monitoring, was carried out at four sampling periods in spring 2020 and 2021: end-March, end-April, early-May and late-May.

1.2. Statistical analyses

Abundance of mummies (unhatched + hatched) and hoverfly eggs (unhatched + hatched) were analysed using GLMM. A negative binomial link was used to account for overdispersion. The models were as follows:

$$\text{Variable tested} \sim \text{variety} + \text{clover} + \text{year} + \text{variety} \times \text{clover} + \text{variety} \times \text{year} + \text{clover} \times \text{year} + (1 | \text{sampling period}) + (1 | \text{site})$$

2. Results

2.1. Diversity and abundance of aphid foliar enemies

Over the two years of monitoring conducted in spring, parasitoid mummies (unhatched or hatched) represented the dominant aphid foliar enemies on barley tillers, representing 63% of the total counts. Then, hoverfly was the second most numerous aphid foliar enemy, whose most represented form was eggs (unhatched or hatched) representing 24% of total counts. Other aphid foliar enemies such as ladybirds or lacewings were poorly represented, regardless of their development stage (Table 5.5).

In spring 2020, a total of 252 mummies (unhatched+hatched) were counted. On these same tillers, a total of 1617 aphids were counted (see chapter 5 part 1), the overall parasitism rate was thus 13%. In spring 2021, a total of 537 mummies (unhatched+hatched) were counted. On these same tillers, a total of 8728 aphids were counted, the overall parasitism rate was thus 6%.

Table 5.5. Aphid foliar enemies observed on barley tillers in spring in the two years of monitoring.

Aphid foliar enemy	Stage	Year *		Total *
		2020	2021	
Mummy	unhatched	114 (21%)	386 (54%)	500 (40%)
	hatched	138 (25%)	151 (21%)	289 (23%)
Hoverfly	egg unhatched	51 (9%)	78 (11%)	129 (10%)
	egg hatched	157 (29%)	15 (2%)	172 (14%)
	larva	58 (11%)	35 (5%)	93 (7%)
	pupa	15 (3%)	12 (2%)	27 (2%)
Ladybird	egg	1 (0%)	19 (3%)	20 (2%)
	larva	0 (0%)	19 (3%)	19 (2%)
	pupa	2 (0%)	1 (0%)	3 (0%)
	adult	2 (0%)	0 (0%)	2 (0%)
Lacewing	egg	4 (1%)	4 (1%)	8 (1%)
	Total	542	720	1262

* Total number of individuals per taxon and relative proportion (in brackets) for each year of monitoring and for both years combined. n=3600 tillers observed in 2020 and n=2880 tillers observed in 2021.

2.2. Temporal dynamic of aphid foliar enemies

At the first monitoring date, at the end of March, the counts of mummies and of hoverfly eggs, both hatched and unhatched, were non-null (Figure 5.8). Then, these numbers increased during the spring. For the mummies unhatched, the maximum counts occurred in the late April sampling period in 2020 (very slight peak), and in the early May sampling period in 2021 (Figure 5.8a). The maximum number of hatched mummies was delayed to the following sampling period (early May in 2020 and late May in 2021). For hoverfly eggs, the maximum number of hatched eggs (end of April in both years) also occurred one sampling period before the maximum number of unhatched eggs (Figure 5.8b). There was a high increase in hatched hoverfly eggs in the early May sampling period in 2020.

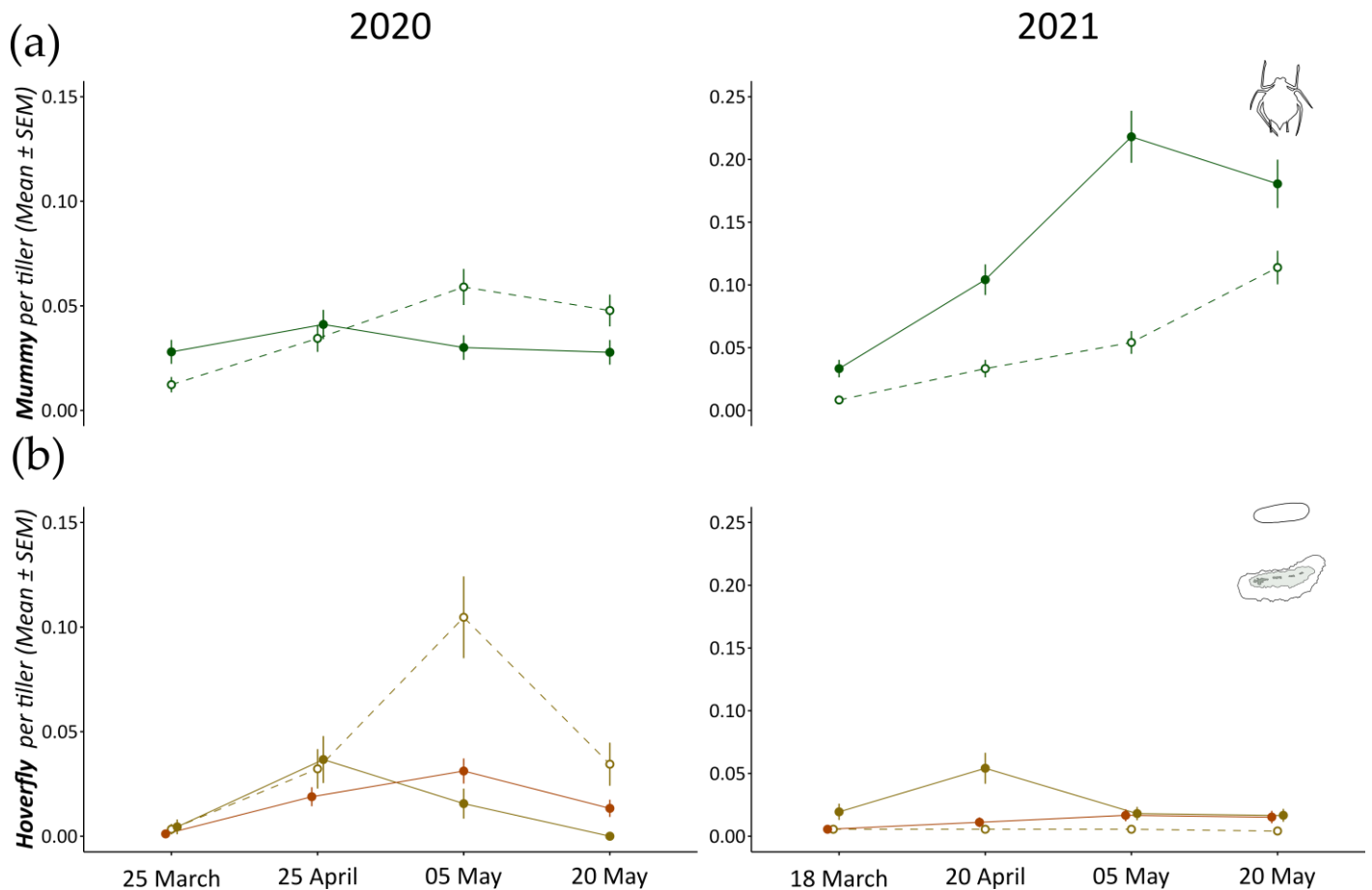


Figure 5.8. Monitoring of aphid foliar enemies on barley tillers in spring. Abundance per tiller (Mean \pm SEM) of: **(a) Parasitoid mummy:** unhatched (—●—) and hatched (---○---) and **(b) Hoverfly:** egg unhatched (—●—); egg hatched (---○---) and larva (—●—). Per sampling period: n=900 tillers observed in 2019 n=720 in 2020.

2.3. Effects of plant diversification on aphid foliar enemies

Concerning the mummy abundance per tiller, only the combined intra and inter-specific plant diversification had an effect on, but reducing it. Indeed, the *variety* × *clover* interaction was significant ($p=0.04$, Table 5.6), and it can be observed that, in 2020, the mummy abundance in the mixture/clover treatment was lower than in the mixture treatment, and it was also lower than in the Amistar/clover and Margaux/clover treatments (Figure 5.9a). The *year* factor was also significant ($p=0.007$), with mummies being two times more abundant in 2021 compared to 2020 (Figure 5.9a).

Concerning the hoverfly egg abundance per tiller, intra-specific diversification had no effect on it, although the factor *variety* was significant ($p=0.04$, Table 5.6). Indeed, the abundance in the mixture was lower than the abundance in Margaux, but equivalent to the one in the Amistar treatment. Inter-specific plant diversification had an effect on hoverfly egg abundance. Indeed, the factor *clover* was significant ($p < 0.001$), abundance being globally reduced in the presence of clover. The *clover* × *year* interaction was also significant ($p < 0.001$), hoverfly egg abundance being decreased in presence of clover in 2020 (reduction of 61%) and only slightly or not reduced in 2021 (Figure 5.9b).

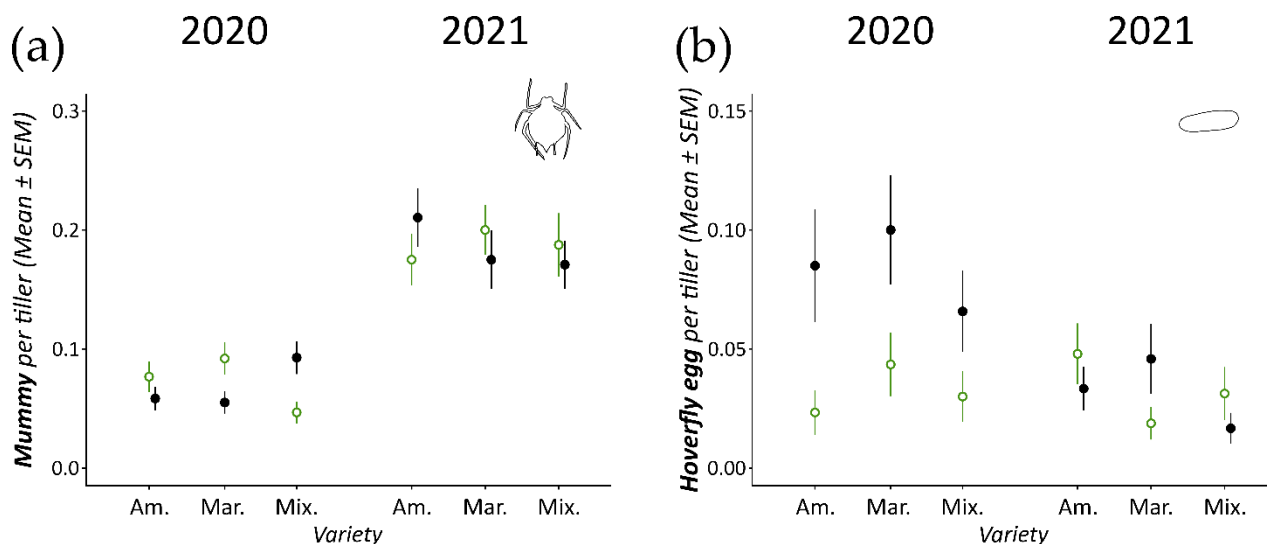


Figure 5.9. Abundance of aphid foliar enemies in spring regarding variety mixture or sole variety and barley intercropped or not with clover. Mean ± SEM per tiller of: **(a)** Parasitoid mummy (unhatched + hatched) and **(b)** Hoverfly egg (unhatched + hatched). Per treatment: $n = 600$ tillers observed in 2020 $n = 480$ in 2021.

Treatment with clover (○); Treatment without clover (●).

Table 5.6. Statistical test results on the effect of different factors and their interactions on aphid foliar enemy populations in spring.

	p-value *						R ² **	
	V	C	Y	V × C	V × Y	C × Y	R ² m	R ² c
Parasitoid mummy (unhatched+hatched)	0.87	0.82	0.007	0.04	0.80	0.81	0.02	0.07
Hoverfly egg (unhatched+hatched)	0.04	< 0.001	0.21	0.30	0.16	< 0.001	0.009	0.05

V: variety; C: clover; Y: year; V × C: interaction between variety and clover; V × Y: interaction between variety and year; C × Y: interaction between clover and year. * p-values from GLMM models. P-values < 0.05 are written in bold.

** R²m: marginal R-squared value considering only the variance explained by the fixed effects; R²c: conditional R-squared value considering the variance explained by both fixed and random effects.

3. Discussion

3.1. Aphid foliar enemies active in spring

Both hoverflies and parasitoids were active in spring. Parasitism by parasitoids was higher in 2020 (13%) than in 2021 (6%).

3.2. Plant diversification can reduce aphid foliar enemy populations

Mummy abundance was reduced when the two types of plant diversification were combined in 2020. In addition, the same year, hoverfly eggs were less abundant on barley when it was intercropped with clover. A first hypothesis to explain this reduction would be that a higher plant diversity could disturb the parasitoid or the adult hoverfly in its search for aphids (disturbing visual, tactile, chemical cues used) (Sheehan, 1986), decreasing their egg laying. Species identification of parasitoids could permit to precise this effect of plant diversification. For example, by analysing whether specialist and generalist species are equally impacted by plant diversification (Jeavons, 2020). A second hypothesis would be that adults laid the same number of eggs, but that hoverflies eggs or mummies were more consumed by other arthropods in the more diversified treatments. In the presence of clover, in spring 2020 (see chapter 5 part 1), ground-dwelling arthropods were more abundant, and could consume hoverfly eggs or mummies (Snyder and Ives, 2001). Thus, further studies on food webs in a such diverse plant system would be necessary. A last hypothesis to explain these reductions in the diversified modalities would be related to the suppressive effect of clover on weeds. Indeed, in the trial, clover showed a significant effect in reducing weed biomass, reducing it by half (biomass at barley harvest, Grauby *et al.*, not published). Thus, in the clover modalities, fewer flowering weeds in spring could explain lower levels of foliar enemies, which adults can feed on.

Although some aphid foliar enemies were active during the two springs, the presence of clover did not increase their populations. However, the clover cover did not flower during the period (with the exception of *Trifolium incarnatum*, which was flowering at the end of April 2020) (see chapter 5 part 1). A flowering companion plant during the spring period could provide pollen and floral nectar to aphid foliar enemies, whose adults are obligatory consumers of these resources (Wäckers, 2005). Indeed, the longevity, fecundity and dispersion of these foliar enemies is highly dependent on the consumption of these resources (Leatemia *et al.*, 1995; Jervis *et al.*, 1996; Wäckers, 2003). Thus, the adaptation of this intercropping practice to foliar enemies could be considered, by proposing species that flower from April to May, whose floral nectaries and pollen are accessible to the targeted foliar enemies (Wäckers, 2004).

Part 3. Synthesis of the spring results

The figure 5.10 presents in a figure form the results of all statistical analyses of this chapter 5. The first, second and third columns represent respectively the effect of intra-specific, inter-specific and both types of diversification combined on all biological parameters monitored.

Concerning intra-specific diversification, this type of diversification did not decrease aphid populations. Indeed, it would have required a decrease in populations in the mixture compared to those in Amistar and in Margaux (which would be indicated as follows in the figure: “↘ mix vs. mar and ↘ mix vs. am”), which is never the case. This type of diversification has no effect on the aphids' enemies, either ground-dwelling or foliar.

Concerning inter-specific diversification, it is clear that this type of plant diversification had the greatest effect on the parameters observed. For aphids, the effect is different depending on the species considered. Clover x variety interactions were observed for *S. avenae* and *R. padi*, indicating that the effect of clover alone was not as clear as for *M. dirhodum*. There is also an effect of clover which increases the activity-density of ground-dwelling predators of aphids in 2020, with a greater effect on ground beetles than on spiders or rove beetles. The opposite effect is observed in 2021 with a decreased activity of ground predators in the presence of clover. No effect of clover on mummy abundance was observed. Clover decreases the abundance of hoverfly eggs in 2020.

Regarding the combination of the two types of diversification, this practice does not allow to observe a synergy, i.e. lower aphid populations in this modality compared to all other modalities. If it was the case, lower aphid populations would have been observed in the mixture/clover modality compared to the mixture alone (“↘ mix.C vs mix”). Lower populations would also have been observed in the mixture/clover modality compared to Amistar/clover and Margaux/clover (“↘ mix.C vs am.C and ↘ mix.C vs mar.C”). This is never the case. This type of plant diversification had no effect on ground-dwelling aphid predators, nor on hoverflies. In 2020, this type of diversification decreased the abundance of mummies.
















		Spring							
		intra		inter		intra + inter			
		2020	2021	2020	2021	2020	2021		
Abundance	Cereal aphids	All sp.	∅		∅		∅		
		<i>S. a</i>	↘ mix vs. mar (mix ~ am)	↗ mix vs. am (mix ~ mar)	↗ am.C vs. am ↗ mar.C vs. mar	↘ mar.C vs. mar ↘ mix.C vs. mix	∅	↘ mix.C vs. mix (mix.C ~ am.C)	
		<i>R. p</i>	↗ mix vs. mar (mix ~ am)	∅	↘ mix.C vs. mix	↘ mar.C vs. mar	↘ mix.C vs. mix (mix.C ~ mar.C)	∅	
		<i>M. d</i>	∅	∅	x0.8	↘	∅	∅	
	Foliar enemies		↘ mix vs. mar (mix ~ am)		x0.4	↘	∅	∅	
			∅		∅		↘ mix.C vs. mix ↘ mix.C vs. am.C / mar.C	∅	
	Ground dwelling arthropods		All sp.	∅		x1.5	↗	x0.6	↘
			<i>A. d</i>	∅		x2	↗	x0.7	↘
			<i>P. c</i>	∅		x2.7	↗	x0.5	↘
			All fam	∅		∅	∅	∅	∅
			Lyc	∅		x1.4	↗	x0.7	↘
			Lin	∅		∅	∅	∅	∅
	∅		x1.2	↗	∅		∅		
Occurrence	Cereal aphids	All sp.	↗ mix vs. am (mix ~ mar)	↗ mix vs. mar (mix ~ am)	∅	∅	∅	∅	
		<i>S. a</i>	↗ mix vs. am (mix ~ mar)	↗ mix vs. am ↗ mix vs. mar	↗ am.C vs. am ↗ mar.C vs. mar	↘ mar.C vs. mar ↘ mix.C vs. mix	∅	↘ mix.C vs. mix (mix.C ~ am.C ~ mar.C)	
		<i>R. p</i>	∅		∅		∅		
		<i>M. d</i>	∅		x0.9	↘	∅		

Figure 5.10. Synthesis of the spring results of the field experiment. Effects of different types of diversification (intra-specific, inter-specific and both combined) on:


Abundance of: cereal aphids (). All sp. = all species combined, S. a = *Sitobion avenae*, R. p = *Rhopalosiphum padi*, M. d = *Metopolophium dirhodum*; hoverfly eggs (hatched + unhatched) (); mummies (hatched + unhatched) (); ground beetles (). A. d = *Anchomenus dorsalis*, P. c = *Poecilus cupreus*; spiders (). All fam = all families combined, Lyc = Lycosidae, Lin = Linyphiidae; and rove beetles ().

The occurrence of cereal aphids ().

Plant diversification may increase () , decrease () or have no effect () on abundance/occurrence. A green or red arrow indicates respectively a positive or negative effect of plant diversification on observed parameters in the sense of biological regulation (i.e. green which decreases the pest and increases its natural enemies and red the opposite).

am = Amistar variety, **mar** = Margaux variety, **mix** = mixture.

am.C = Amistar variety intercropped with clover.

NB: " mix vs. mar" means "decreased abundance in the mixture compared to the Margaux variety alone".

"mix ~ am" means "equivalent abundance in the mixture and in the Amistar variety".

" am.C vs. am" means "decreased abundance in Amistar/clover compared to the Amistar variety alone".

In this case, statistical analyses showed a significant clover x variety interaction. If the year is specified, there is also a significant clover x year interaction. If there is only an arrow in the box (common to both years) it means that only the clover factor is significant, without interaction. In this case "x 2" indicates that the abundance/occurrence is doubled in the presence of clover.

CHAPITRE 6

EFFET DE LA DIVERSIFICATION VEGETALE INTRA-PARCELLAIRE SUR LE RENDEMENT DE L'ORGE ET SES DIFFERENTES COMPOSANTES



Chapitre 6. Effet de la diversification végétale intra-parcellaire sur le rendement de l'orge et ses différentes composantes

Intra-field plant diversification: impact on grain cereal yield and on the different yield components

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Keywords: Intercropping; cultivar mixture; clover; winter barley; yield; competition

Abstract: Intra-field plant diversification practices can be considered as multi-services. Cereal/legume intercropping can, among others, increase nitrogen supply for the following crop, increase weed control and reduce pests and diseases. Cereal varieties mixture, can decrease diseases and allow a better weed control. But for the large-scale implementation of these practices, their impact on yield must be studied. In this regard, we investigate the effects of the use of barley variety mixture and intercropping with clover on barley yield and its components. Field trials were set up on nine organic commercial farms in South-East of France during the 2019-2020 and 2020-2021 growing seasons. In June, at the cereal harvest, quadrats of the different crops were harvested, dried and weighed. Different yield components were calculated: the spike number per square meter, number of grains per spike, the 1000-grain weight, the grain yield. The biomass of barley, clover and weeds were also monitored. Clover had a significant negative impact on cereal yield, reducing it by 16% on average. The analysis of the different yield components showed a competition of the clover at different periods of the barley growing cycle: stem extension stage, flowering stage and grain filling stage. However, clover showed a strong reduction in weed biomass, reducing it by 50% on average. Concerning intra-specific diversification, the yield of the mixture was not increased compared to the yield of the pure varieties.

1. Introduction

Increasing diversity within cultivated fields can be an alternative to conventional farming practices and the use of insecticides. Indeed, in many situations, this practice enhances pest control and promotes the presence of natural enemies (Altieri and Nicholl, 1994; Gurr *et al.*, 2004). However, for a broader development and a sustainable use of these practices in agroecosystems by farmers, the link between diversification practices and crop yield needs to be assessed (Gurr and Wratten, 1999).

Some studies have investigated the impact of inter-specific crop diversification on yield. In their literature review based on analyses of 58 field experiments, Bedoussac *et al.* (2015) found that cereals intercropped with legumes showed i) higher yield (22% increase on average) and more stable grain yield than in sole crop, ii) higher cereal protein content (13% augmentation) than in sole crop, and iii) higher and more stable gross margin than in sole crop (22% higher). Verret *et al.* (2017) conducted a meta-analyse on intercropped system with legumes and found a higher yield of intercropped crop compared to sole crop in 57% or 44% of the studies (non-weeded and weeded sole crop, respectively). Moreover, the "win-win" situation i.e. where the yield was higher and weed biomass decreased, represented 52% and 36% of the studies (non-weeded and weeded sole crop,

respectively). Several mechanisms are involved into the reduction of weed biomass associated with a yield increase: i) competition mechanisms (where the companion plant competes with weeds and occupies their ecological niche. Once the weeds suppressed, the soil nitrogen is then used for crop biomass production instead of weed biomass, increasing the crop yield), ii) facilitation mechanisms (via nitrogen transfer from the companion plant to the main crop), and iii) allelopathy mechanisms, where the companion plant inhibits weed growth, via the emission of chemical compounds (Liebman and Dyck, 1993).

Concerning intra-specific plant diversification, different meta-analyses reported the potential of cultivar mixtures for increasing crop yield relatively to pure varieties. Smithson and Lenne (1996) found on average a yield increase of 5.4% in cereal mixtures, Kiær *et al.* (2009) a yield increase of 3.9% and Borg *et al.* (2018) an increase of 3.5%. However, it is suggested that this increase in cereal grain yield might be dependant of different factors such as cereal species, the number of varieties and their proportion in the mixture (Kiær *et al.*, 2009). In addition, higher yields were found in mixtures heterogeneous in disease resistance and in phenology (Kiær *et al.*, 2009; Borg *et al.*, 2018) but also in mixtures that allow a better competition with weeds (Kiær *et al.*, 2009).

In an on-farm field experiment testing two types of plant diversification i.e. intra-specific diversification via a barley varieties mixture and inter-specific diversification with barley intercropped with clover, as well as both types of diversification combined, the following hypotheses were tested:

- 1) Varieties mixture (intra-specific plant diversification) shows a slightly better yield than single varieties.
- 2) Barley intercropped with clover shows an equivalent or better yield than the barley grown alone. The association also provides better weed control.

2. Materials and methods

2.1. Wheat and clover performance

On the same experimental sites as the field experiment (see chapter 4 and 5), measurements in the 6 treatments were carried out at barley harvest (5 fields in 2020 and 4 fields in 2021). Six samples per treatment were collected using 0.25 m² quadrats positioned along a diagonal transect (Figure 6.1), in which the above-ground plant biomass was hand-harvested and sorted according to crop (barley or clover) and weeds. The collected samples were oven-dried for 48 hours at 80°C and then weighed. For each sample, barley straws and spikes were weighed separately and the number of spikes was determined. Then the spikes were threshed and the grains were weighed. A sub-sample of 350 grains per sample was taken and weighed, in order to calculate the 1000-grain weight.

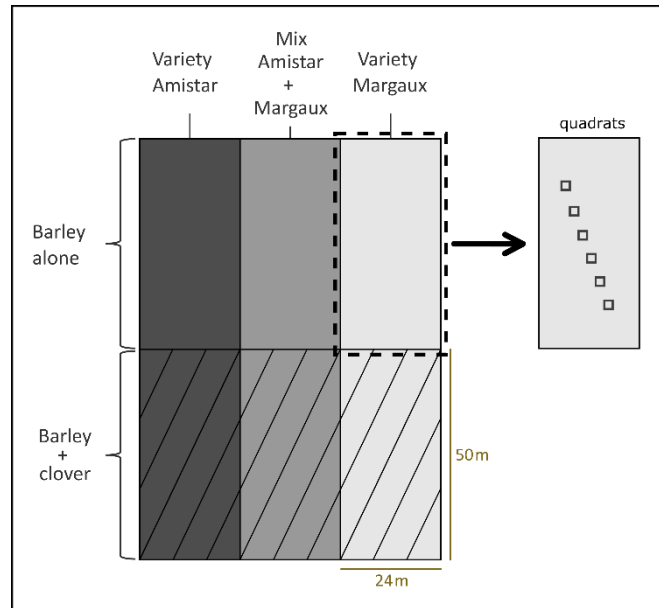


Figure 6.1. Design of the field experiment and monitoring of crop performance with quadrats.

2.2. Statistical analyses

Barley (straw + spike), clover and weed biomass were expressed in dry matter ton per hectare (DMt ha⁻¹). Concerning barley yield and its components, several variables were computed. Grain yield (in t ha⁻¹) and 1000-grain weight (in gram) were calculated and adjusted to 15% moisture (Vrignon-Brenas *et al.*, 2018). Spike number per square meter and theoretical grain number per spike were also calculated.

These variables were analysed using Generalized Linear Mixed Effects Models (GLMM). The factor *variety* (3-levels factor: Amistar, Margaux and Mixture), the factor *clover* (2-levels factor: with clover and without clover), the factor *year* (2-levels factor: 2020 and 2021), and their interactions were used as fixed effects. A random effect of the site was added.

Variable tested ~ variety + clover + year + variety × clover + variety × year + clover × year + (1 | site)

A Gaussian family logit link was used for the different variables. The significance of fixed factors and their interaction was determined by a likelihood ratio test (LRT). Statistical analyses were performed using R version 4.1.1 with lme4 package for GLMM. drop1 function was used to perform LRT.

3. Results

The statistical tests showed fairly similar effects of the tested factors on the different variables of yield, its components and on barley biomass (Table 6.1). On all these variables, the factor *variety* and the *variety* × *year* interaction were significant (except for the factor *variety* which was not significant for the spike number per m²). While in 2020 Amistar showed similar performances as Margaux or the mixture, in 2021 its yield, its different components, as well as barley biomass were lower than the ones of Margaux or the mixture (Figure 6.2a, 6.2b, 6.2c, 6.2e). Intra-specific diversification did not lead to an increase of the different variables compared to the varieties alone.

The factor *clover* appeared significant for all these variables: consistently, in the presence of clover, yield, its components and barley biomass were decreased. The barley yield was significantly decreased by 16% on average in presence of clover (yield reduced by 11% in 2020 and 19% in 2021)

($p < 0.001$, Table 6.1, Figure 6.2a). Clover significantly decreased the spike number by 8% ($p < 0.05$, Figure 6.2b) and the 1000-grain weight by 3% ($p < 0.001$, Figure 6.2d) for each year of monitoring. The barley biomass was also decreased by 13% in presence of clover (11% reduction in 2020 and 14% in 2021).

For the grain number per spike, the *clover* \times *year* interaction was also significant ($p = 0.04$), in link with a higher decrease in the grain number per spike in the presence of clover in 2021 than in 2020 (reduction by 3% in 2020 and by 11% in 2021) (Figure 6.2c). It can be noted that the *variety* \times *clover* interaction was never significant for the different parameters. Combining the two types of diversification had no effect on the yield and its components.

For the clover biomass, the factor *variety* and the *variety* \times *year* interaction were almost significant ($p = 0.07$). Graphically, we observe a lower clover biomass in the Margaux treatment in 2021 compared to the mixture and Amistar, which was not the case in 2020 where all three were equivalent (Figure 6.2f).

For the weed biomass, only the factor *clover* was significant ($p < 0.001$). The presence of clover resulted in a 51% reduction in weed biomass on average compared to the treatments without clover (reduction by 48% in 2020 and 57% in 2021) (Figure 6.2g).

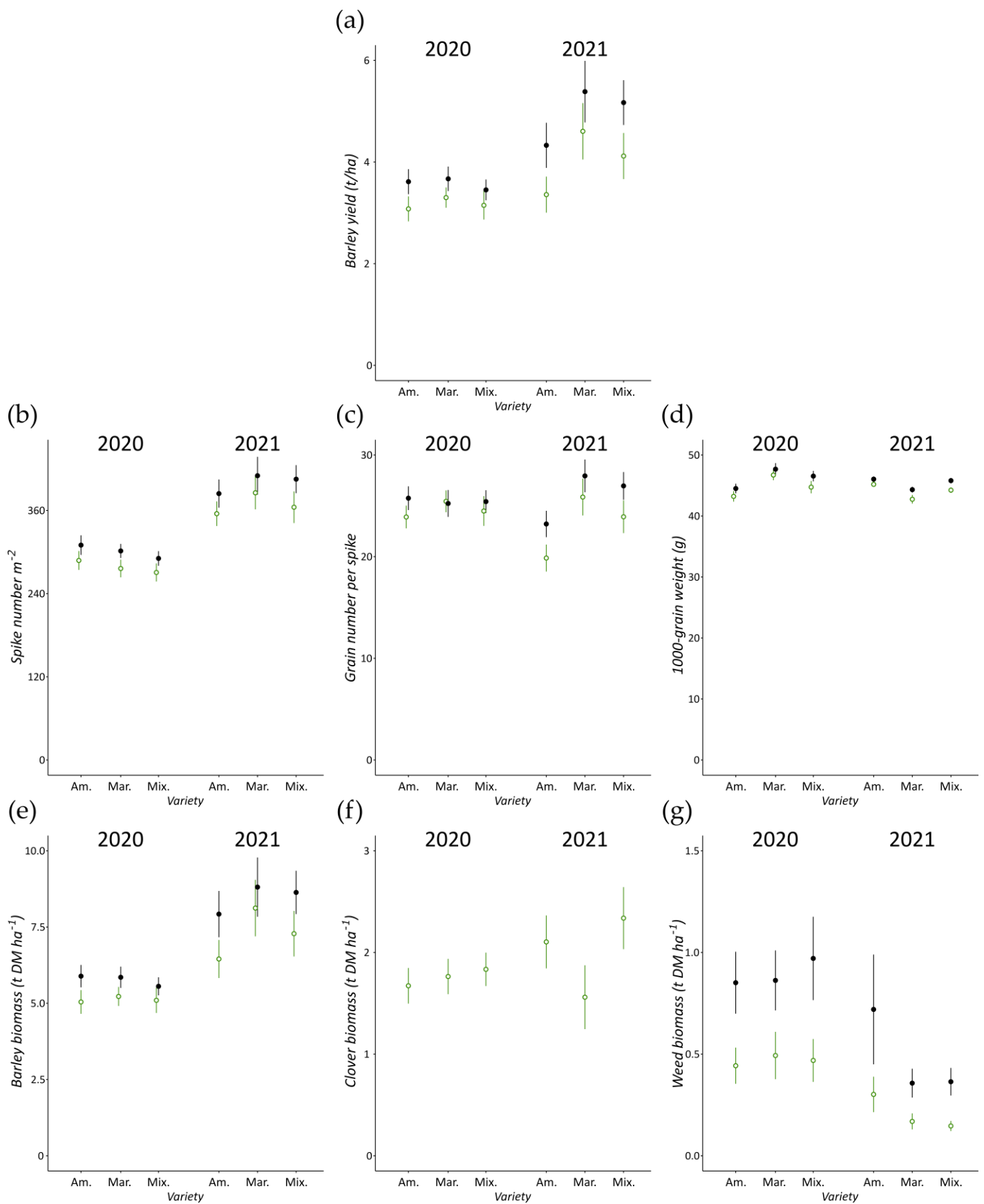


Figure 6.2. Biomass, barley yield and its components regarding variety mixture or sole variety and barley intercropped or not with clover. (a) Barley grain yield; (b) Spike number per square metre; (c) Grain number per spike; (d) 1000-grain weight; (e) Barley biomass; (f) Clover biomass; (g) Weed biomass. Mean \pm SEM (n=30 in 2020 and n=24 in 2021). Treatment with clover (○); Treatment without clover (●).

Table 6.1. Statistical test results on the effect of three factors and their interaction on plant biomass, barley yield and its components.

Variable tested		Year	Without clover *			With clover *			p-value **						R ² ***	
			Amistar	Margaux	Mixture	Amistar	Margaux	Mixture	V	C	Y	V × C	V × Y	C × Y	R ² m	R ² c
Barley yield and its components	Yield (t ha ⁻¹) 15% moisture	2020	3.6 ± 0.2	3.7 ± 0.2	3.4 ± 0.2	3.1 ± 0.2	3.3 ± 0.2	3.1 ± 0.3	< 0.001	< 0.001	0.29	0.81	< 0.001	0.02	0.10	0.77
		2021	4.3 ± 0.4	5.4 ± 0.6	5.2 ± 0.4	3.4 ± 0.4	4.6 ± 0.6	4.1 ± 0.5								
	Spike number m ⁻²	2020	310.0 ± 14.4	301.6 ± 10.4	290.8 ± 10.8	287.9 ± 13.9	276.3 ± 13.0	270.5 ± 13.3	0.44	< 0.001	0.05	0.96	0.04	0.51	0.21	0.67
		2021	384.3 ± 20.8	410.2 ± 27.9	405.2 ± 20.7	355.3 ± 18.1	385.5 ± 24.4	364.7 ± 23.3								
	Grain number per spike	2020	25.8 ± 1.2	25.2 ± 1.3	25.4 ± 1.1	23.9 ± 1.1	25.4 ± 1.1	24.5 ± 1.5	< 0.001	< 0.001	0.91	0.34	< 0.001	0.04	0.03	0.68
		2021	23.2 ± 1.3	27.9 ± 1.6	27.0 ± 1.4	19.9 ± 1.4	25.9 ± 1.9	23.9 ± 1.6								
1000-grain weight (g) 15% moisture	2020	44.5 ± 0.8	47.7 ± 1.0	46.5 ± 0.9	43.2 ± 0.9	46.7 ± 0.9	44.7 ± 1.0	0.02	< 0.001	0.70	0.64	< 0.001	0.97	0.04	0.70	
	2021	46.0 ± 0.5	44.3 ± 0.5	45.8 ± 0.5	45.2 ± 0.4	42.7 ± 0.7	44.2 ± 0.5									
Biomass (DMt ha ⁻¹)	Barley	2020	5.9 ± 0.4	5.9 ± 0.4	5.6 ± 0.3	5.0 ± 0.4	5.2 ± 0.3	5.1 ± 0.4	0.02	< 0.001	0.17	0.56	0.02	0.14	0.14	0.78
		2021	7.9 ± 0.8	8.8 ± 1.0	8.6 ± 0.7	6.5 ± 0.6	8.1 ± 0.9	7.3 ± 0.8								
	Clover	2020	/	/	/	1.7 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	0.07	/	0.66	/	0.07	/	0.02	0.52
		2021	/	/	/	2.1 ± 0.3	1.6 ± 0.3	2.3 ± 0.3								
	Weeds	2020	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.43	< 0.001	0.26	0.70	0.07	0.22	0.10	0.48
		2021	0.7 ± 0.3	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.1 ± 0.0								

V: factor variety (Amistar, Margaux, Mixture); C: factor clover (without or with clover); Y: factor year (2020 or 2021); V × C: interaction between the factor variety and the factor clover; V × Y: interaction between the factor variety and the factor year; C × Y: interaction between the factor clover and the factor year.

* Mean ± SEM of each of the 6 treatments tested in the field experiment.

** p-values from GLMM models. P-values < 0.05 are written in bold.

*** R²m: marginal R-squared value considering only the variance explained by the fixed effects; R²c: conditional R-squared value considering the variance explained by both fixed and random effect

4. Discussion

4.1. Contrasted yields between years and varieties

Barley yields were higher in 2021 than in 2020. One first possible explanation for this difference is the climatic conditions, especially water availability. Indeed, the spring 2020 was dry and hot with important water deficit towards the heading stage of barley, while the spring 2021 was cooler and rainier (see chapter 5 part 1). Another possible explanation is the higher weed biomass on field trials in 2020 with almost 1t DM/ha (treatments with cereal grown alone), that could have competed more strongly with the cereal (personal communication, Florian Celette).

In 2021, the variety Amistar showed lower yield performances compared to Margaux and particularly for the grain number per spike. The Margaux variety may have traits (e.g. disease resistance, development rate, *etc.*) more adapted than those of Amistar to the conditions of the year.

In 2021, the varieties mixture showed results closer to those of Margaux than the ones of Amistar. The Margaux variety may have prevailed over Amistar in the mixture. However, this could not be confirmed through visual monitoring during crop development, as the two varieties were very similar visually. In contrast to other studies, the yield of the mixture was not increased compared to the yield of the pure varieties (Smithson and Lenne, 1996; Kiær *et al.*, 2009; Borg *et al.*, 2018).

4.2. Clover negatively impacted cereal yield and its components

Clover had a significant negative impact on cereal yield (16% reduction on average), which represents a substantial economic loss for farmers. The analysis of the different yield components showed a competition of the clover at different periods of the barley growing cycle.

The spike number per square meter was the component the most impacted by the presence of clover in both years (8% reduction). This yield component is related to three cereal growing stages: cereal emergence (i.e. number of plants per m²), tillering and stem extension stages. However, the clover cover was poorly developed during the barley emergence in autumn and during the barley tillering in winter (see chapter 4 part 1). Thus, these results suggest that the clover competed with the cereal rather at the end of the winter or at the beginning of the spring (stem extension stage). The number of grains per spike is the yield component related to the flowering/pollination stages of the cereal (occurring in early May in the present study). The reduction recorded suggests that the clover competition with the barley may have been low in 2020 (3% reduction) and higher in 2021 (11% reduction). However, the ground cover data do not show a difference in clover cover between the two years at this period (40% of ground cover on average, see chapter 4 part 1). Since the ground cover was estimated visually, a more accurate measurement at this period such as clover biomass might have provided a better understanding of this competition.

The last yield component, the 1000-grain weight is related to the grain filling stage of the cereal (occurring in late May in the present study). The competition of clover with barley at this stage may have occurred, but was low (3% reduction). Thus, grain filling was marginally impacted by clover in both years of monitoring.

Clover has thus competed with barley at different stages of its growing cycle, with competition decreasing towards the end of the cycle. The competition between clover and cereal for resources can occur for light and soil resources such water and nutrients (Malézieux *et al.*, 2009). Competition for water certainly occurred, especially in the spring 2020 which was particularly dry in the study region. Although the legume is able to fix the atmospheric nitrogen, competition for soil nitrogen between the

cereal and the legume is possible, especially in low-nitrogen soils (Vrignon-Brenas *et al.*, 2018) and could also have occurred in our field trials, all conducted in organic farming.

4.3. Clover cover successfully regulates weeds

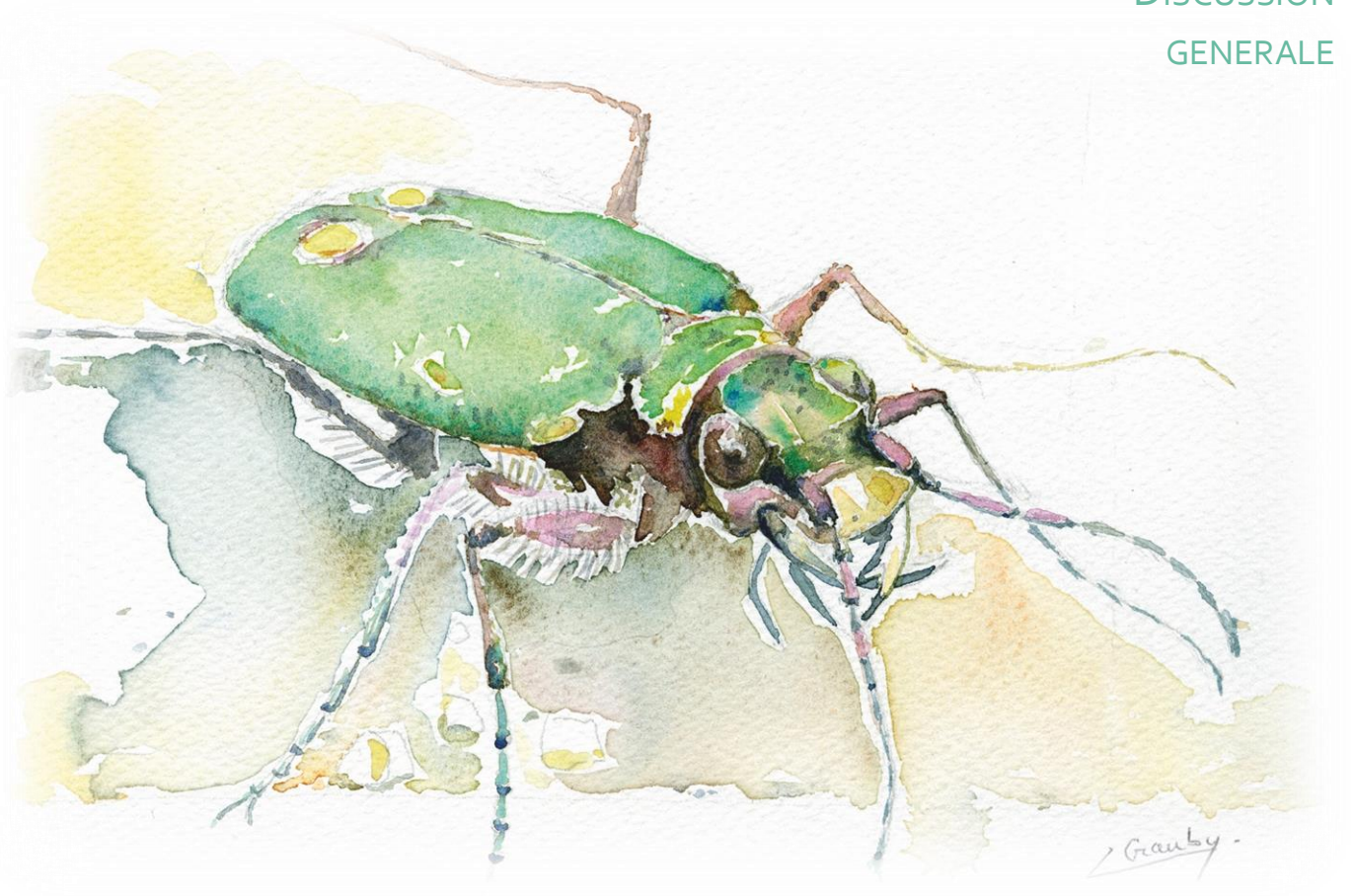
The clover ensured a very effective regulation of the weed flora, reducing their biomass at harvest by half, in both years of monitoring. This service provided by the companion plant represents a real benefit for farmers, particularly in organic farming. It should be noted that no weed control was applied in the clover treatments of the trial. In the barley alone treatments, 6 trials out of 9 were weeded (one harrowing pass between November and March). Thus, by associating barley with clover, weeds are better controlled, resulting in human work and energy (fuel) savings and in a reduction of soil compaction risk. In addition, after the cereal harvest, the clover (especially white clover in the present study), if undestroyed, can continue to cover the soil in the summer and autumn and therefore limit soil erosion, weed infestation (Vrignon-Brenas *et al.*, 2018). During this period, the clover also continues to fix atmospheric nitrogen, which returns to the soil and to the next crop after destruction.

4.4. Possible options to limit the clover-cereal competition

The present study showed that the clover cover caused strong competition with the weeds, but also with the main crop. In the meta-analysis conducted by Verret *et al.* (2017), this “lose-win situation” in presence of legume (yield and weed biomass decreased) concerned 30% of the studies. For a successful implementation of the practice in cropping systems, options to limit the negative effect of the competition are therefore needed. Several levers could be activated for this purpose such as the sowing density or the plant composition of the cover crop. In our case, the clover sowing density (13kg/ha), could be reduced and adjusted depending on the site (especially on poor, superficial soils). In addition, the proportion between the different clover species (5 kg/ha of *Trifolium alexandrinum*, 5 kg/ha of *Trifolium incarnatum* and 3 kg/ha of *Trifolium repens*) could have been adjusted differently. For example, the *T. incarnatum* was particularly well developed in the spring (when the cereal-clover competition was high, see chapter 5 part 1), its proportion could therefore be reduced, and the proportion of *T. repens*, which was less developed in spring, could be increased. Other legume species, with a lower biomass, could also be considered for the association. However, this needs to be considered in relation to the aphid (and the weed) regulation service for which a higher biomass of the companion plant would have a greater effect.

CHAPITRE 7

DISCUSSION GENERALE



Aquarelle, Cicindèle champêtre
Cicindela campestris
Christine Grauby

Chapitre 7. Discussion générale

Partie 1. Pucerons des céréales et dégât indirect sur stades précoces de la céréale

1. Résultats en conditions contrôlées : dispositif en arène

Le dispositif en arène multi-plantes a permis de tester à l'échelle de la population de pucerons (deux générations produites au bout de 13 jours) l'effet d'une plante non-hôte sur plusieurs paramètres liés au vecteur et au virus. Le design expérimental (densité de semis de l'orge et du trèfle, arrangement spatial des cultures) a été choisi de manière à ressembler le plus possible aux paramètres de la culture de ces plantes en conditions réelles au champ. Deux morphes de pucerons fondateurs ont été testés.

1.1. Puceron fondateur de morphe ailé

La présence de trèfle modifie la répartition spatiale des populations de pucerons quand l'individu fondateur est de morphe ailé. En effet, les populations de pucerons qui se répartissent en deux foyers distants aux deux extrémités de l'arène en absence de trèfle, passent à une répartition en un seul foyer en présence de trèfle. Cet effet spatial du trèfle n'est détecté que dans le cas d'un individu fondateur ailé : pour le morphe aptère, les populations de pucerons sont distribuées en un seul foyer. Puisque les descendants sur deux générations de l'individu fondateur (ailé ou aptère) sont de morphe aptère, l'effet spatial du trèfle résulte d'un effet de cette plante non-hôte sur l'individu fondateur ailé lui-même. L'effet spatial du trèfle sur ce fondateur ailé pourrait s'expliquer par une perturbation du trèfle sur sa mobilité initiale, qui semble relativement importante. En effet, la distance entre la plante de dépôt et la plante fondatrice est multipliée par deux quand l'individu fondateur est ailé. Puis, les deux foyers créés aux deux extrémités de l'arène par l'ailé en absence de trèfle, indique également une mobilité initiale importante de ce dernier. Enfin, des éléments bibliographiques indiquent que l'individu ailé est capable de « vols triviaux » testant plusieurs plantes distantes avant de choisir une plante pour s'installer de manière plus sédentaire (Moericke, 1955). Ainsi, l'expérimentation en arène indique une perturbation de la mobilité initiale de l'ailé en présence de trèfle, réduisant ainsi le nombre de foyers créés. Si cet effet du trèfle se retrouvait en conditions au champ, il serait très intéressant pour réduire le nombre de points d'introduction d'ailés dans la parcelle, évènement pouvant mener à l'infection primaire de la maladie.

1.2. Puceron fondateur de morphe aptère

1.2.1. Une réduction des populations de pucerons en présence de trèfle

Une réduction des populations de pucerons en présence de trèfle a été observée quand l'individu fondateur est de morphe aptère. Après 13 jours d'expérimentation, les deux paramètres abondance et occurrence des pucerons par plante sont diminués en présence de la plante non-hôte. Les mécanismes sous-jacents pourraient être de plusieurs types : le trèfle pourrait représenter une gêne visuelle, olfactive et/ou physique pour le puceron (Perrin et Phillips, 1978; Finch et Collier, 2000; Randlkofer *et al.*, 2010; Xie *et al.*, 2012; Ninkovic *et al.*, 2013). Dans sa recherche de plante hôte, le puceron teste les plantes avec lesquelles il rentre en contact grâce à ses antennes et en sondant les cellules de l'épiderme (piqûres d'essais). Dans une situation de plantes associées, le puceron peut passer une grande partie de son temps et de son énergie à tester des plantes non-hôtes, au détriment de la colonisation et de l'alimentation sur sa plante-hôte (Hooks et Fereres, 2006). Une réduction plus importante de l'abondance (réduction de 21% et 27% pour la densité 1 et 2 de trèfle) par rapport à

l'occurrence (réduction de 6% et 18%) a été observée (Figure 7.1). Ce résultat pourrait s'expliquer par la dynamique exponentielle des populations de pucerons, où un nombre très important d'individus est produit sur une période temporelle courte. En 13 jours, 350 pucerons en moyenne ont été produits à partir d'un seul individu fondateur aptère, avec seulement 36 plantes à leur disposition dans le dispositif en arène. Ainsi, le paramètre abondance de pucerons par plante peut être plus facilement diminué par la présence d'une plante non-hôte que le paramètre occurrence de pucerons par plante où un seul puceron suffit pour coloniser une plante.

1.2.2. Une prévalence de la maladie inchangée en présence de trèfle

Bien qu'en présence de trèfle, les populations de pucerons, vecteurs du virus, soient diminuées, la prévalence de la maladie, i.e. l'occurrence du virus par plante, reste inchangée (Figure 7.1). Les théories classiques d'épidémiologie ont fait l'hypothèse simplifiée que la transmission d'un virus dépend exclusivement de l'abondance du vecteur, mais la prise en compte de ce seul paramètre est aujourd'hui considérée comme insuffisante (Strauss *et al.*, 2020). La prise en compte d'autres paramètres tel que la démographie du vecteur et sa dispersion sont essentiels pour expliquer la dispersion d'un virus. De plus, la contribution des différents paramètres à l'épidémiologie d'un virus est variable selon son mode de transmission (Madden *et al.*, 2000; Roudine *et al.*, 2023).

Concernant la densité du vecteur par plante, ce paramètre influencerait de manière limitée l'épidémiologie d'un virus transmis de manière persistante circulante (= circulant non multipliant (Madden *et al.*, 2000; Roudine *et al.*, 2023). En effet, pour ce mode de transmission, qui est celui du BYDV, un seul vecteur peut infecter plusieurs plantes à différents moments de sa vie. Pour impacter de manière significative la dispersion d'un virus transmis de manière persistante circulante, la densité du vecteur devrait être très fortement diminuée (Madden *et al.*, 2000). Dans l'expérimentation en arène conduite dans la thèse, le trèfle a permis une diminution de l'abondance de 21%/27% et de l'occurrence de 6%/18% (respectivement pour la densité 1 et 2 de trèfle). Une première hypothèse pour expliquer l'absence d'effet du trèfle sur la prévalence de la maladie serait une diminution des populations de pucerons (et notamment de l'occurrence de pucerons par plante) insuffisante pour observer une réduction significative de l'occurrence du virus par plante.

Des paramètres relatifs à la fitness du vecteur (fécondité, longévité) sont également importants dans l'épidémiologie d'un virus transmis par vecteur (Figure 7.1). D'après Madden *et al.* (2000), la longévité du vecteur serait un paramètre influençant particulièrement l'épidémiologie d'un virus transmis de manière persistante circulante. En effet, la transmission de virus par ce mode implique des phases d'acquisition, d'inoculation et de latence longues. Une longévité diminuée du vecteur, diminuerait ainsi la probabilité que ces événements se produisent (Madden *et al.*, 2000). Ainsi, une deuxième hypothèse qui permettrait d'expliquer une prévalence inchangée de la JNO en présence de trèfle, est que la plante non-hôte perturberait peu la longévité de *R. padi*. Cette hypothèse pourrait être testée à l'échelle de l'individu non mobile par un dispositif de « clip-cages » installées sur orge associé ou non au trèfle, dans lequel la survie et la fécondité d'un adulte de *R. padi* seraient suivis quotidiennement (Amline *et al.*, 2022).

Enfin, des paramètres concernant le comportement du vecteur (mobilité, comportement alimentaire) sont également essentiels dans l'épidémiologie d'un virus vecté (Madden *et al.*, 2000; Crowder *et al.*, 2019; Strauss *et al.*, 2020) (Figure 7.1). Par exemple, une augmentation de la mobilité d'un vecteur peut augmenter le taux de rencontre vecteur-plante hôte. Si la durée d'alimentation est inchangée, couplé à un taux de rencontre augmenté, la dispersion d'un virus transmis de manière persistante circulante peut être augmentée.

Au contraire, une durée d'alimentation diminuée peut permettre une diminution de la transmission du virus (temps d'acquisition et d'inoculation réduits) (Grilli et Holt, 2000). Ainsi, une troisième hypothèse qui permettrait d'expliquer une prévalence inchangée de la JNO en présence de trèfle, est que la plante non-hôte altérerait peu la mobilité de *R. padi* et/ou la durée d'alimentation du puceron sur sa plante hôte. Des expérimentations complémentaires pourraient être réalisées pour tester cette hypothèse. Par exemple, le comportement alimentaire de *R. padi* sur orge associé ou non au trèfle pourrait être étudié grâce à la technique de l'EPG (Électropénétrographie). En effet, des auteurs ont observé une perturbation de certaines phases alimentaires chez *Myzus persicae* en présence d'une plante non-hôte (Baudry *et al.*, 2021; Ameline *et al.*, 2022). Une modification du comportement alimentaire du vecteur en présence de la plante non-hôte, par exemple au travers d'une perturbation des phases de salivation et de durée d'ingestion phloémienne, aurait potentiellement un impact sur la transmission du virus (ces phases étant responsables respectivement de l'inoculation et d'acquisition du virus BYDV). Un facteur supplémentaire pourrait être également ajouté à cette étude par EPG : le statut sanitaire du puceron (sain ou virulifère) (Chesnais *et al.*, 2020). En effet, la présence de virus dans son vecteur pourrait modifier le comportement de ce dernier en présence d'une plante non-hôte i.e. l'interaction virus-vecteur (Figure 7.1).

Le dispositif en arène multi-plantes a ainsi permis de tester l'effet d'une plante non-hôte sur un virus transmis de manière persistante circulante par puceron. Les résultats obtenus à l'échelle de la population de pucerons, indiquent des pistes qui pourront être approfondies à l'échelle individuelle afin de déterminer si certains paramètres de la biologie du vecteur sont perturbés en présence de trèfle. D'autres expérimentations multi-plantes de plus grande taille pourraient aussi être réalisées, pour confirmer les résultats obtenus.

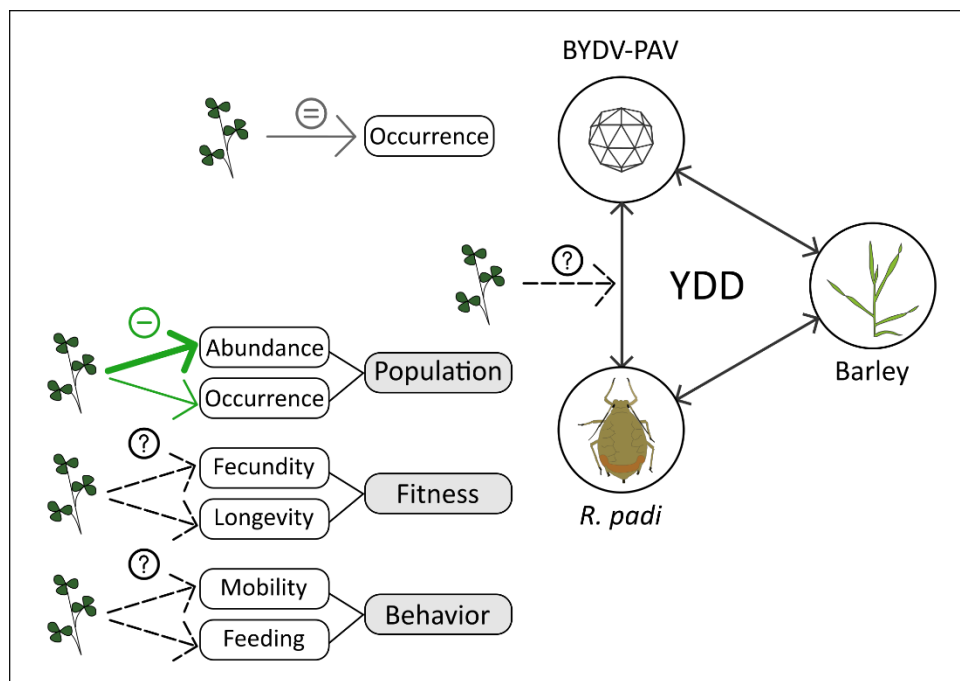


Figure 7.1. Effets du trèfle sur différents paramètres du pathosystème étudié. Résultats de l'expérimentation en arène quand l'individu fondateur est de morphe aptère (⊖ : réduction du paramètre ; ⊖ : paramètre inchangé ; ? : effet sur le paramètre inconnu).

1.3. Propositions d'autres expérimentations en arène

Bien que *R. padi* soit le principal vecteur de la JNO en automne, d'autres espèces de pucerons tel que *S. avenae* peuvent également être présents, et vecteurs du virus, sur les jeunes semis de céréales. Le même dispositif en arène multi-plantes pourrait être testé avec l'espèce *S. avenae*. Cette espèce de puceron est considérée comme étant mobile (Holmes, 1988; Power, 1990) et l'introduction d'une plante non-hôte pourrait exacerber cette mobilité et altérer la dispersion du virus transmis par l'espèce. De plus, la position de *S. avenae* sur la céréale est différente de celle de *R. padi*, la première espèce étant positionné principalement sur les feuilles supérieures (Watt, 1979), tandis que la seconde se situe en position basse, proche du sol, sur les feuilles ou la tige (Leather et Dixon, 1981). Ces deux espèces utilisent probablement en différentes proportions les voies de passages (via le sol, via les feuilles) pour passer d'une plante hôte à une autre et l'effet du couvert de trèfle pourrait donc être différent selon l'espèce de puceron considérée. Enfin, d'autres espèces de virus pourraient également être testées dans ce dispositif multi-plantes, par exemple l'espèce BYDV-MAV dont *S. avenae* est une des principales espèces vectrices. Les temps des différentes phases de vection entre BYDV-PAV transmis par *R. padi* et BYDV-MAV transmis par *S. avenae* pourraient légèrement varier, et par conséquent mener à un effet différencié du trèfle sur la dispersion de ces espèces de virus.

2. Résultats en conditions réelles : terrain d'automne-hiver

2.1. Effet de la diversification végétale sur le pathosystème

Dans l'expérimentation conduite sur le terrain en automne-hiver, les deux types de diversification végétale intra- et inter-spécifique n'ont eu aucun effet significatif sur les populations de pucerons des céréales, que ce soit sur l'arrivée des ailés ou sur la dissémination des aptères dans la parcelle, et ce quel que soit l'espèce de puceron considérée. Un effet légèrement significatif a toutefois été détecté lorsque les deux types de diversification végétale étaient combinées : l'occurrence par plante des pucerons des céréales, toutes espèces confondues, a été diminuée. La diversification végétale n'a par contre pas eu d'effet sur l'abondance des ennemis naturels terrestres des pucerons (carabes, araignées, staphylins) ni sur l'abondance de leurs ennemis foliaires (parasitoïdes, syrphes). Elle n'a également eu aucun effet sur la prévalence de la JNO dans la parcelle.

L'absence d'effet de la diversification intra-spécifique sur les pucerons des céréales pourrait s'expliquer par une concentration en composés organiques volatils insuffisante et diluée au champ par rapport aux conditions de laboratoire. Ainsi, le mécanisme de communication plante-plante à l'origine de l'effet d'un mélange de variété sur les pucerons observé en laboratoire (Shoffner and Tooker, 2013; Pettersson *et al.*, 1999; Ninkovic *et al.*, 2002) ne se produirait pas en conditions réelles de culture.

En ce qui concerne l'absence d'effet de la diversification inter-spécifique, ceci pourrait s'expliquer par un manque de développement du couvert de trèfle en automne-hiver (Figure 7.2). En effet, les services fournis par une plante de service et notamment celui du contrôle des insectes ravageurs, sont fortement et positivement corrélés à la biomasse de la plante de service (Gardarin *et al.*, 2022).

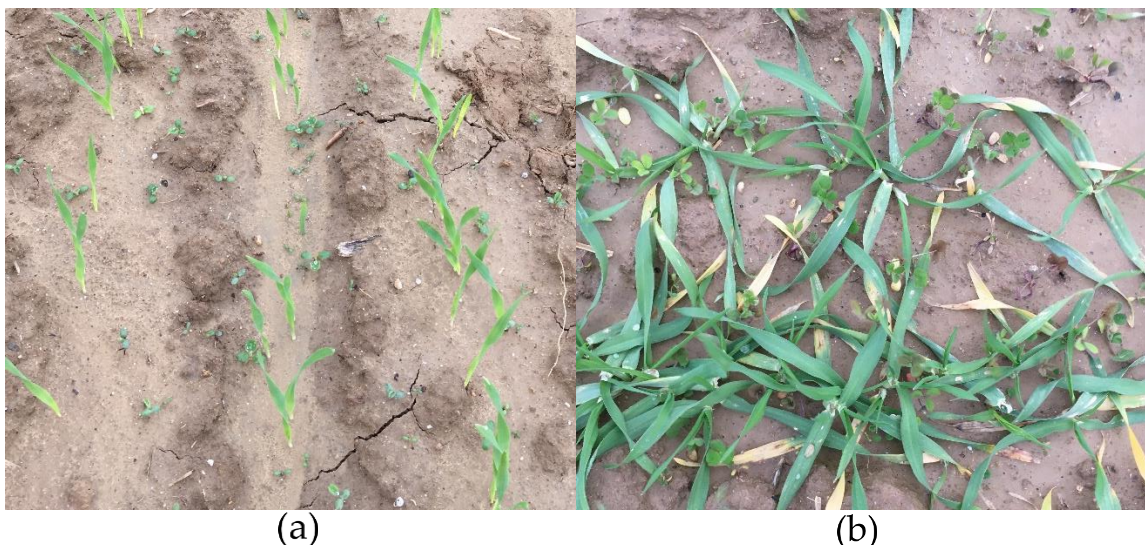


Figure 7.2. « Simultaneous intercropping » : une plante compagne peu développée en automne-hiver. Sur un essai de la thèse semé le 15.10.19 (semis « précoce ») (a) photo prise le 31.10.19 (b) photo prise le 16.12.19.

2.2. Propositions d'optimisation de la pratique d'intercropping en automne-hiver

2.2.1. Espèces de plantes de service

Trois espèces de trèfle en mélange ont été choisies pour constituer le couvert associé à la céréale. Ce mélange a été choisi pour avoir une fonction de couverture du sol sur une grande période du cycle de la céréale, grâce à la complémentarité des cycles des trois espèces (cycle rapide, intermédiaire et long pour respectivement le trèfle incarnat, le trèfle d'alexandrie et le trèfle blanc). Cependant, dans une optique de régulation biologique des pucerons en début de cycle de la céréale, les fonctions recherchées du couvert pourraient être concentrées sur la période automnale/hivernale. Pour chaque fonction recherchée, l'utilisation des traits fonctionnels des espèces végétales pourrait permettre d'identifier des plantes compagnes aux caractéristiques adaptées.

Couverture du sol et production de biomasse

Des traits fonctionnels relatifs à une production de biomasse importante par la plante de service en automne-hiver pourraient être privilégiés, comme par exemple : une vitesse de croissance rapide et un taux de couverture élevé du sol en automne-hiver ainsi qu'une résistance au froid. Une analyse bibliographique de la littérature scientifique et celle d'organismes techniques pourrait être effectuée afin d'établir une liste d'espèces végétales ayant ces traits fonctionnels. Par exemple, le trèfle souterrain, le trèfle de Perse et le trèfle de Micheli pourraient être de bons candidats car ils ont une implantation rapide et une bonne couverture du sol en automne-hiver (Enache et Ilnicki, 1990; Vial Prairies, communication personnelle, 2023)(Figure 7.3).

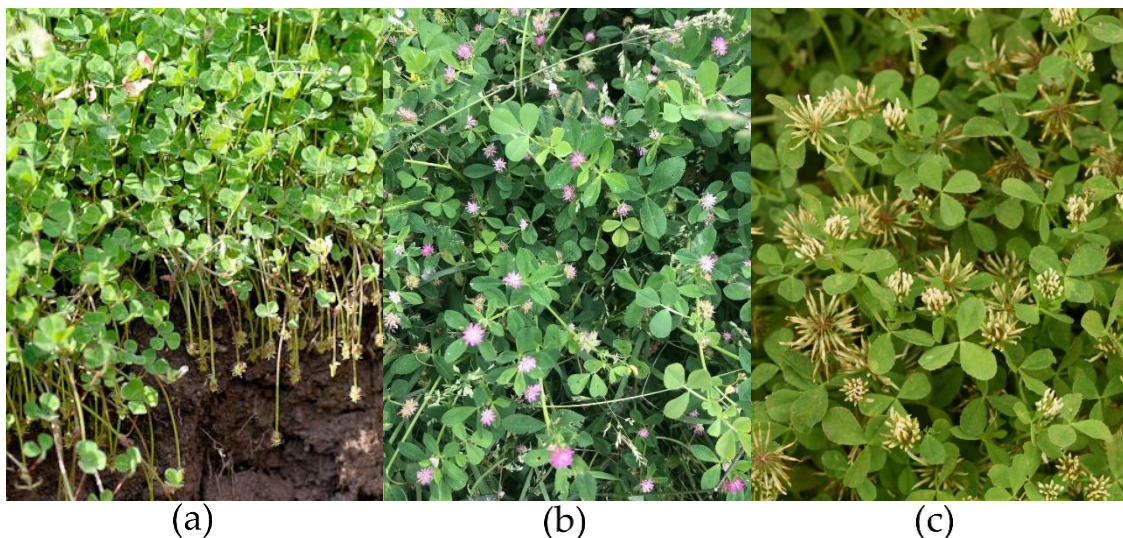


Figure 7.3. Espèces de trèfle à implantation rapide et ayant une bonne couverture du sol en automne. (a) Trèfle souterrain, *Trifolium subterraneum* (Source photo : FloreAlpes, 2010) ; **(b)** Trèfle de Perse, *Trifolium resupinatum* (Source photo : Alex D., 2022) ; **(c)** Trèfle de Micheli, *Trifolium michelianum* (Source photo : Gilli C., 2019).

Floraison

Des traits fonctionnels relatifs à la floraison de la plante de service pourraient également être pris en compte afin d'attirer les ennemis foliaires des pucerons (syrphes, parasitoïdes). Ces traits seraient par exemple une floraison en automne-hiver (Figure 7.4) et la fourniture de pollen/nectar accessibles aux différents ennemis foliaires des pucerons (Figure 7.4b). Cependant, le trait de floraison en automne-hiver semble difficilement compatible avec un semis simultané de la plante de service avec la céréale en octobre. Ainsi, si cette fonction de la plante de service devait être favorisée, d'autres types d'arrangements temporels (e.g. semis sous « living mulch », voir la partie 2.2.3 de cette partie) ou spatiaux (e.g. bandes fleuries autour de la parcelle) devraient être envisagés.

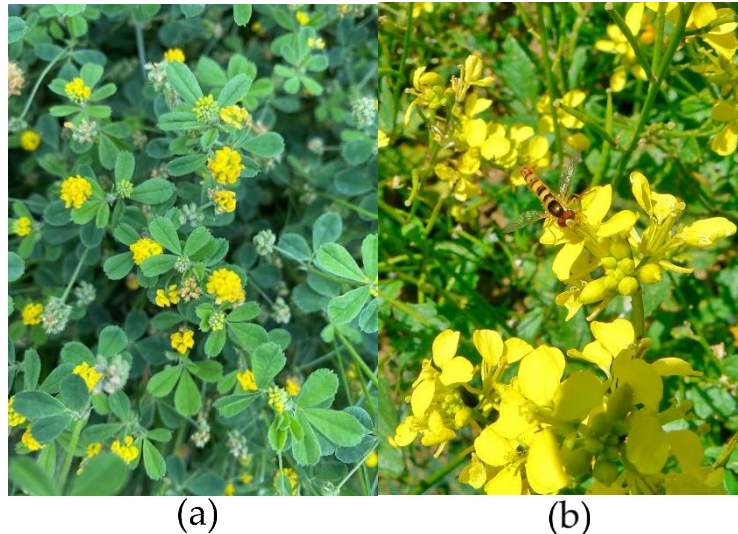


Figure 7.4. Exemple d'espèces végétales pouvant fleurir en automne-hiver. (a) Luzerne lupuline (Wallace et Scott, 2008) (Source photo : Laming D., 2020) ; **(b)** Moutarde blanche (*Sinapis alba*) et Syrphide porte-plume (*Sphaerophoria scripta*) dont les larves sont aphidiphages (Source photo : Geslin B., 2020).

Propriétés répulsives

Enfin, des traits fonctionnels correspondant à une fonction répulsive de la plante de service vis-à-vis des pucerons pourraient être envisagés. Dans leur review, Ben Issa *et al.* (2017a) ont identifié des études reportant des effets répulsifs de plantes de services sur des espèces de pucerons. Cependant dans ces études, les plantes hôtes sont majoritairement des cultures légumières ou arboricoles. De plus, les plantes non-hôtes sont des plantes appartenant aux familles botaniques des Alliées (ail, oignon, ciboulette) (Amarawardana *et al.*, 2007; Lai *et al.*, 2011; Potts et Gunadi, 1991; Sarker *et al.*, 2009), Lamiacées (basilic, romarin, lavande, sarriette) (Ben Issa *et al.*, 2017b; Basedow *et al.*, 2006; Beizhou *et al.*, 2010) ou Astéracées (souci officinale, œillet d'inde) (Jankowska *et al.*, 2009; Feng et Yang, 2011). Or même si ces plantes présentaient des effets répulsifs également sur les pucerons des céréales, elle seraient cependant difficiles à mettre en place en association avec des grandes cultures. En grande culture, et notamment en culture de céréales, l'association la plus utilisée est celle avec des plantes appartenant à la famille des légumineuses (Fabacées), notamment pour leur capacité à fixer l'azote atmosphérique. Les genres les plus représentés parmi les légumineuses sont les genres *Medicago* (e.g. la luzerne), *Vicia* (e.g. la vesce commune) et *Trifolium* (i.e. les trèfles) (Gardarin *et al.*, 2022). Ainsi, il serait pertinent d'identifier si les espèces déjà utilisées en association en grande culture possèdent des propriétés répulsives contre les pucerons des céréales. En parallèle, il serait intéressant d'effectuer une analyse de la littérature scientifique afin de déterminer si d'autres espèces auraient ce trait fonctionnel, et qui seraient compatibles à une utilisation en grande culture.

Ainsi, une analyse bibliographique ciblant des espèces végétales possédant certains traits fonctionnels (couverture du sol rapide/importante, floraison en automne-hiver, propriétés répulsives vis-à-vis des pucerons) permettrait de produire une liste d'espèces candidates. Ces espèces pourraient ensuite être testées sur le terrain (« étape de screening »), en les semant en automne et en suivant leur développement pendant la période hivernale. En parallèle, ces espèces pourraient être testées en conditions de laboratoire vis-à-vis de leur potentiel de régulation des pucerons des céréales (particulièrement sur l'espèce *R. padi*). Enfin, dans une dernière étape, les espèces présentant les meilleurs résultats pourraient être testées en association avec une céréale, et des suivis de populations de pucerons, de leurs ennemis naturels et de prévalence de la JNO pourraient être réalisés.

2.2.2. Arrangement spatial des plantes associées

Dans l'expérimentation terrain de la thèse, l'arrangement spatial le plus simple techniquement à mettre en place a été utilisé : le « mixed intercropping » de l'orge et du trèfle. D'autres arrangements spatiaux pourraient être testés, tel que le « row » ou le « strip intercropping » avec un ou plusieurs rangs de céréale, en alternance avec un ou plusieurs rangs de la plante compagne (Figure 7.5). Cette pratique, aurait un bon potentiel dans la lutte contre les ravageurs des cultures, en augmentant la complexité de l'environnement dans lequel l'insecte ravageur recherche sa plante-hôte (Landis *et al.*, 2000), la bande de plante non-hôte représentant une barrière physique entravant les déplacements de l'insecte entre les rangs (Mollaie *et al.*, 2021) puis en fournissant un habitat pour ses ennemis naturels (Snyder, 2019). Cette pratique permettrait également une meilleure optimisation de l'utilisation des ressources par les plantes (liée à leur plus grande séparation dans l'espace) ce qui stabiliserait les rendements (Du *et al.*, 2018; Raseduzzaman et Jensen, 2017). Cette pratique, déjà testée en culture de céréale (blé associé à du colza ou de la luzerne), a montré des effets prometteurs pour diminuer les populations de *S. avenae* (Ma *et al.*, 2007; Nassab *et al.*, 2013; Alarcón-Segura *et al.*, 2022). Cependant, les suivis ont été réalisés au printemps, avec des cultures potentiellement bien développées et avec une présence importante d'ennemis naturels. Les effets de ce type d'arrangement spatial sur les populations de pucerons des céréales en automne-hiver restent toutefois méconnus. Ainsi, il serait intéressant de tester le potentiel de cette pratique pour réguler les populations de pucerons et diminuer la prévalence de la JNO sur le terrain, en faisant varier le nombre de rangées de chacune des cultures. Différentes espèces de plantes de service pourraient également être testées dans cet arrangement spatial (voir la partie 2.2.1 de cette partie).



Figure 7.5. « Strip intercropping » : rangs alternés d'orge et de trèfle blanc. (Source photo : Banfield-Zanin *et al.*, 2021).

2.2.3. Arrangement temporel des plantes associées

Afin que le couvert de trèfle soit plus développé en automne-hiver et donc que la biomasse soit plus importante, un semis anticipé de ce dernier, avant le semis de l'orge, pourrait être envisagé. Cette pratique qualifiée de « living mulch » (semis sous « paillis vivant ») consiste à semer une culture de rente dans une plante de service déjà établie (Figure 7.6). Le living mulch peut être utilisé pour de nombreux services (réduction du besoin en travail du sol, réduction de l'érosion et du lessivage des nitrates, etc.), mais le service le plus recherché est celui du contrôle des adventices, cette pratique d'intercropping étant la plus efficace pour les contrôler (Bhaskar *et al.*, 2021; Gardarin *et al.*, 2022). L'effet d'un living mulch sur la régulation d'insectes ravageurs a été étudié principalement sur la culture de légumes (chou, concombre, courgette, etc.). Les études ont montré des résultats prometteurs pour diminuer l'abondance des ravageurs, voir pour attirer leur ennemis naturels (Altieri *et al.*, 1985; Andow *et al.*, 1986; Costello, 1994; Frank et Liburd, 2005; Kahl *et al.*, 2019). Toutefois l'effet d'un living mulch sur les insectes ravageurs en grandes cultures reste méconnu. Cette pratique a bien été testée en culture de céréale d'hiver mais pour la fourniture d'autres services, tel que le service de production. Thorsted *et al.* (2006) puis Hiltbrunner et Liedgens (2008) ont expérimenté le semis d'un blé d'hiver dans un couvert de trèfle blanc déjà établi depuis le printemps. Pour limiter la concurrence du trèfle avec la culture principale, risque majeur avec ce type de pratique, les auteurs ont testé une gestion mécanique du couvert de trèfle. Lors du semis du blé, un cultivateur rotatif a été utilisé pour labourer des bandes dans le couvert de trèfle pour y semer le blé. Puis des coupes de trèfle (d'une à trois coupes) entre les rangs de céréales, ont été réalisées d'avril à mai. Des augmentations de rendement et de la concentration en azote dans les grains grâce aux coupes du trèfle ont été observées. Ainsi, cette pratique de living mulch pourrait être testée en parcelles expérimentales en automne-hiver. Un couvert de trèfle pourrait être semé au printemps/été avant la céréale d'hiver, semée en automne. Si cette pratique montrait des résultats prometteurs pour réguler les populations de pucerons des céréales et la JNO, il y aurait cependant un besoin de développement d'outils pour gérer mécaniquement le couvert à grande échelle, accessibles et utilisables par les agriculteurs (Thorsted *et al.*, 2006).



Figure 7.6. « Living mulch » : avoine semé sous un couvert déjà établi de trèfle. Photo prise en novembre (Source photo : Amos D., 2021).

Une pratique d'intercropping peut ainsi être conduite au travers de divers arrangements spatiaux et temporels au champ. Ces pratiques ont principalement été étudiées dans la littérature scientifique pour leur effet sur la fourniture d'azote, le rendement ou sur la régulation des adventices. Leur effet sur la régulation biologique des ravageurs est moins étudié. Lorsque l'effet sur la régulation des ravageurs a été étudié, le potentiel de certaines plantes de services pour réguler les ravageurs au champ, a surtout été évalué sur des espèces végétales choisies initialement pour d'autres services. Or les effets d'une plante de service sur un insecte ravageur sont beaucoup moins génériques que ceux d'une plante de service sur par exemple les adventices, ce qui explique le manque de résultats opérationnels à ce jour (Gardarin *et al.*, 2022). Ainsi, pour chaque ravageur ciblé, le choix de la plante de service et son arrangement temporel et spatial devrait être spécifiquement considéré.

Ainsi, les pratiques visant à augmenter la biomasse de la plante de service, identifiée comme un des leviers principaux pour augmenter la régulation biologique d'un insecte ravageur, devraient être testées spécifiquement sur les pucerons des céréales. Les pratiques identifiées (arrangements spatiaux, arrangements temporels) pourraient être testées au préalable en conditions contrôlé (e.g. par dispositif multi-plantes en arène), puis en conditions réelles au champ pour les pratiques les plus prometteuses. Une analyse socio-économique sur l'utilisation de ces pratiques par les agriculteurs pourrait être réalisée.

Partie 2. Pucerons des céréales et dégâts directs au printemps

1. Résultats du terrain de printemps

1.1. Effet de la diversification intra-spécifique

La diversification intra-spécifique n'a pas permis de diminuer les populations de pucerons des céréales, contrairement aux hypothèses formulées. Ce type de diversification n'a eu aucun effet sur les populations des ennemis naturels des pucerons, qu'ils soient terrestres ou aériens.

1.2. Effet de la diversification inter-spécifique

Parmi les types de diversification végétale testés, c'est la diversification inter-spécifique qui a montré le plus d'effets sur les variables suivies lors du terrain de printemps.

1.2.1. Pucerons

La diversification inter-spécifique a eu des effets significatifs sur les populations de pucerons des céréales, les effets étant variables selon l'espèce de puceron considérée. Les effets les plus nets ont été détectés sur *M. dirhodum* : la présence de trèfle permet une diminution de son abondance et de son occurrence par plante. Pour *S. avenae* et *R. padi*, les effets du trèfle sont moins nets mais montrent cependant une tendance : l'abondance et l'occurrence de *S. avenae* tendent à être augmentés et l'abondance de *R. padi* diminuée en présence de trèfle. Ces effets devraient être confirmés par des expérimentations au champ supplémentaires.

Ces effets différenciés du couvert de trèfle selon l'espèce de puceron, pourraient s'expliquer par leur position différente sur la céréale, générant des différences dans leurs déplacements ou dans leur prédation par les ennemis naturels. *S. avenae* occupant une position haute sur la céréale (Watt, 1979), serait moins accessible et moins consommé par ses prédateurs terrestres, qui peuvent être plus nombreux en présence de trèfle. *S. avenae* pourrait également utiliser une voie de passage inter-plantes différente de celle des deux autres espèces. En effet, en émettant l'hypothèse que *S. avenae* utilise la voie « aérienne » pour passer d'une plante à l'autre, le trèfle, en augmentant les contacts plante-plante, pourrait faciliter les déplacements de l'espèce. À l'inverse, *R. padi* et *M. dirhodum* occupant des positions plus basses sur la céréale (Leather et Dixon, 1981), seraient plus exposés à la prédation par les arthropodes terrestres et potentiellement plus perturbés par l'effet barrière du trèfle en se déplaçant d'une plante à une autre par la voie « terrestre ».

Dans la littérature, les différentes voies de passage des espèces de pucerons des céréales sont méconnues. Au cours d'un stage de M2 réalisé à l'ISARA ces hypothèses de voies de passage différenciées des espèces de pucerons des céréales ont donc été testées. Un protocole expérimental en laboratoire a été développé, présentant une plante donneuse et une plante receveuse, ainsi que quatre modalités où les pucerons peuvent passer : uniquement par le sol (A), par le sol et les feuilles (B), uniquement par les feuilles (D), ou sont bloqués sur la plante donneuse et ne peuvent pas passer (C) (Figure 7.7). Trois espèces ont été étudiées : *R. padi*, *S. avenae* et *Rhopalosiphum maidis* (l'espèce *M. dirhodum* n'ayant pas été obtenue pour débiter un élevage en amont du stage). Cette expérimentation a permis de confirmer l'hypothèse de passage inter-plante de *S. avenae* principalement par la voie foliaire. Concernant les deux autres espèces, les résultats ont été moins nets, notamment à cause de difficultés liées au protocole expérimental pour ces deux espèces (production d'individus ailés, production d'un très grand nombre d'individu sur le temps de l'expérimentation, etc.) (Esteves de Oliveira, 2022).

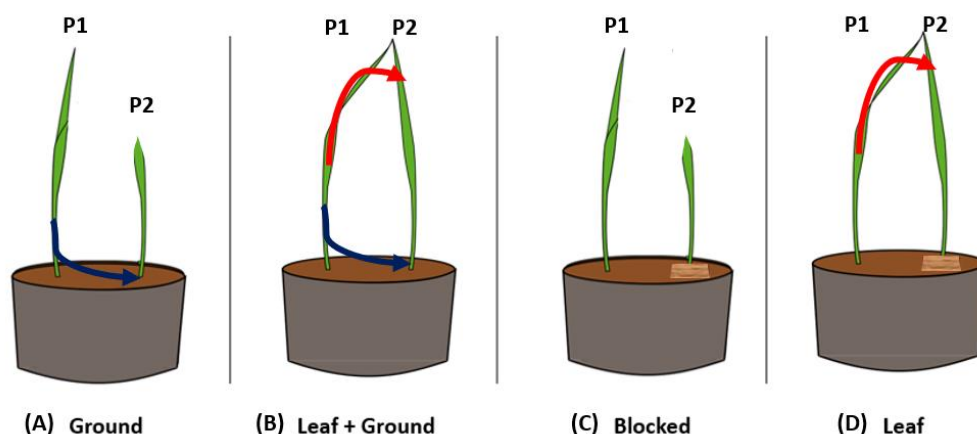


Figure 7.7. Dispositif expérimental à 4 traitements utilisé pour tester les voies de passage inter-plante de trois espèces de pucerons des céréales. (Source : Esteves de Oliveira, 2022).

Ainsi, un couvert de plante non-hôte pourrait ne pas avoir un seul effet générique sur les pucerons des céréales, mais des effets différenciés selon l'espèce considérée. Cet aspect de la diversification inter-spécifique devrait être creusé au travers d'expérimentations complémentaires.

1.2.2. Ennemis naturels terrestres

La diversification inter-spécifique a montré des effets significatifs sur les prédateurs terrestres des pucerons, mais avec des sens opposés selon l'année. En 2020 l'activité-densité des carabes et des lycosidae a été augmentée en présence de trèfle, alors qu'elle a été diminuée en 2021. Des conditions climatiques très différentes entre les deux années peuvent expliquer ces résultats. En 2020, pendant la période de suivi, les conditions climatiques étaient chaudes et sèches, le trèfle pouvant représenter un habitat aux conditions plus favorables que le sol nu pour les arthropodes du sol. En 2021, les conditions climatiques étaient froides et humides, le trèfle était donc potentiellement un habitat moins favorable avec des températures plus basses et une humidité plus importante. Ces observations et les mécanismes sous-jacents restent toutefois à confirmer par des expérimentations complémentaires. De plus, le lien entre l'augmentation de la présence de prédateurs du sol et l'augmentation de la consommation de pucerons reste encore trop peu étudié. Dans des dispositifs expérimentaux comme ceux de la thèse, des analyses de contenus stomacaux pourraient donc être pertinentes (Chen *et al.*, 2000; Kamenova *et al.*, 2018).

1.2.3. Ennemis naturels foliaires

Les syrphes et les parasitoïdes ont été les ennemis foliaires des pucerons les plus comptabilisés lors des suivis effectués sur la céréale. Les autres ennemis foliaires (coccinelles, chrysopes) étaient présents en très faible nombre. La pratique de diversification inter-spécifique n'a pas permis d'augmenter l'abondance totale des syrphes et des parasitoïdes, contrairement aux hypothèses formulées initialement.

Tout d'abord, il se pourrait que l'association de la céréale et du trèfle ait eu un effet mais que le protocole expérimental choisi n'ait pas permis de le détecter. En effet, pour les parasitoïdes, seul un dénombrement du nombre de momies sur orge a été effectué sans identification à l'espèce. Or il se pourrait que le couvert de trèfle ait eu des effets différenciés selon l'espèce de parasitoïde considérée, comme cela a été observé pour les espèces de pucerons hôtes. Concernant les syrphes et plus globalement les prédateurs foliaires des pucerons (coccinelles, chrysopes), un comptage visuel sur orge n'est pas forcément le protocole le plus adapté pour suivre les formes adultes. D'autres

protocoles comme un comptage visuel sur transects, des aspirations ou des captures au filet auraient peut-être été plus pertinents.

De même le potentiel du couvert de trèfle à fournir des proies/hôtes alternatifs n'a pas été évalué. La plante compagne peut héberger des insectes phytophages, dont certains ennemis naturels sont communs aux pucerons des céréales. Par exemple, certaines espèces de parasitoïdes sont capables de parasiter à la fois les espèces de pucerons présentes sur légumineuses (*Aphis craccivora*, *Aphis fabae* et *Acyrtosiphon pisum*) et celles présentes sur les céréales (Luquet, 2019). Par exemple, *Aphidius avenae* et *Aphidius ervi* sont capables de parasiter *S. avenae*, *M. dirhodum* et *R. padi* mais aussi *Acyrtosiphon pisum* (Luquet, 2019). Ainsi, le trèfle, grâce à la fourniture d'hôtes alternatifs, aurait pu favoriser certaines espèces de parasitoïdes, communes aux pucerons des céréales. Des suivis d'ennemis naturels sur le trèfle auraient pu permettre de confirmer ou non cette hypothèse.

Une deuxième hypothèse pourrait être formulée face au résultat obtenu : il n'y a pas d'effet de la diversification végétale inter-spécifique sur les ennemis foliaires. Cette absence d'effet pourrait être due aux caractéristiques de la plante non-hôte. En effet, le mécanisme d'augmentation des populations d'ennemis foliaires grâce à la diversification inter-spécifique, repose sur la fourniture de ressources alimentaires par la plante non-hôte, et notamment de ressources sucrées, que les formes adultes des ennemis foliaires consomment (syrphes, parasitoïdes, chrysopes). Ces ressources sucrées peuvent être du nectar floral, du nectar extrafloral et du miellat, sachant que le nectar est considéré comme la ressource glucidique possédant la meilleure qualité nutritive (Wäckers, 2001). Dans la thèse, seul le trèfle incarnat en 2020 a fleuri au moment du pic de pucerons des céréales, en avril. Les autres espèces (trèfle d'alexandrie et trèfle blanc) et le trèfle incarnat en 2021, ont fleuri après que l'orge soit rentré en sénescence. Ainsi, le couvert de trèfle n'a pas, ou peu, fourni de nectar floral pour les ennemis foliaires des pucerons pendant la période de présence des pucerons. De plus, les espèces du genre *Trifolium* utilisées dans l'essai ne produisent pas de nectar extrafloral (Weber *et al.*, 2015). Or cette ressource sucrée, par rapport au nectar floral, est produite en plus grande quantité, est plus facile d'accès et disponible pendant une plus longue période (Wäckers, 2005). Ainsi, le choix d'un autre genre de légumineuse, tel que le genre *Vicia* (Figure 7.8) pourrait permettre la fourniture de nectar extrafloral sur une grande période et donc potentiellement augmenter le contrôle biologique des pucerons. Toutefois, il est à noter que l'hypothèse d'approvisionnement en nectar (Heimpel et Jervis, 2005) qui prédit que la complémentation en nourriture mène à l'augmentation du contrôle biologique des ravageurs, n'est pas toujours vérifiée (Luquet, 2019).

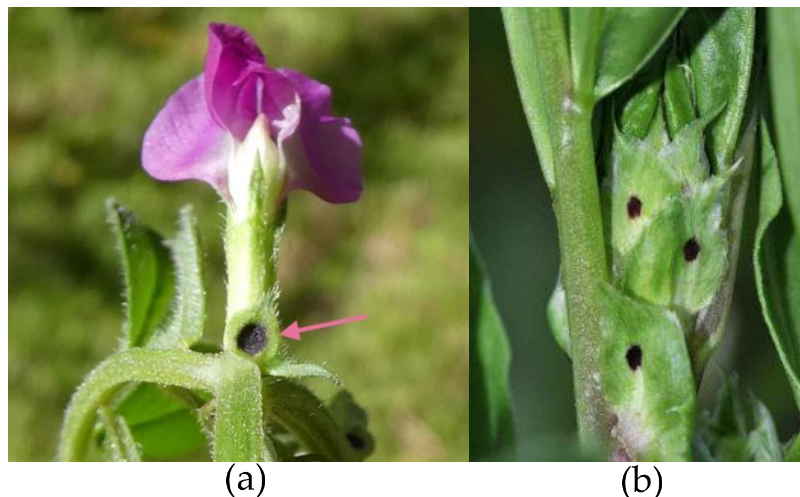


Figure 7.8. Exemple d'espèces possédant des nectaires extra-floraux. (a) *Vicia sativa* (Source photo : Llandrich A., 2020) ; (b) *Vicia faba* (Source photo : Zoom-nature).

1.3. Effet des deux types de diversification combinés

La combinaison des deux types de diversification végétale n'a pas permis de diminuer les populations de pucerons des céréales d'une manière plus importante que dans chaque type de diversification seule. L'hypothèse de « stacking » prédisant un effet de synergie n'est ainsi pas validée par les expérimentations de cette thèse, comme elle ne l'a pas été non plus dans l'expérimentation de Mansion-Vaquié et *al.* (2019).

2. Conclusion

La pratique de diversification végétale intra-parcellaire a été étudiée dans la thèse, sous l'angle de son potentiel de régulation des ravageurs des cultures. Parmi les trois types de diversification végétale testés, seule la diversification inter-spécifique a montré des effets sur les pucerons des céréales et leurs ennemis naturels. Au printemps, ce type de diversification a montré une tendance pour diminuer les populations de *M. dirhodum* et *R. padi*. Mais elle a aussi montré une tendance pour augmenter les populations de *S. avenae*, espèce qui peut provoquer des pertes de rendements au printemps. En automne-hiver, quand la céréale est la plus sensible aux dégâts indirects causés par les pucerons des céréales, vecteurs de la JNO, la pratique d'intercropping n'a pas permis de diminuer les populations de pucerons des céréales, ni de diminuer la prévalence de la maladie. Ainsi, même si ce type de diversification a montré le plus de résultats dans la thèse par rapport à la diversification intra-spécifique ou les deux types de diversification combinés, elle reste à être améliorée pour augmenter ses effets sur les pucerons des céréales. L'expérimentation en laboratoire réalisée dans la thèse, ainsi que d'autres études, indiquent qu'augmenter la biomasse de la plante non-hôte permettrait d'augmenter la régulation biologique des pucerons (par des mécanismes bottom-up et top-down). Cependant, augmenter la biomasse, et notamment en automne, impliquera plus d'opérations à réaliser pour l'agriculteur (e.g. semis en deux temps des cultures, arrangement spatial plus complexe à semer, gestion du couvert par désherbage, etc.) et donc un investissement en temps et financier plus conséquent qu'un semis en simultané sans arrangement spatial particulier. De plus, il faudrait confirmer que les pratiques augmentant la biomasse de la plante non-hôte en début de cycle permettent de diminuer la prévalence de la maladie en automne-hiver, avant que celle-ci soit déployée à plus large échelle sur le terrain.

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Annexes

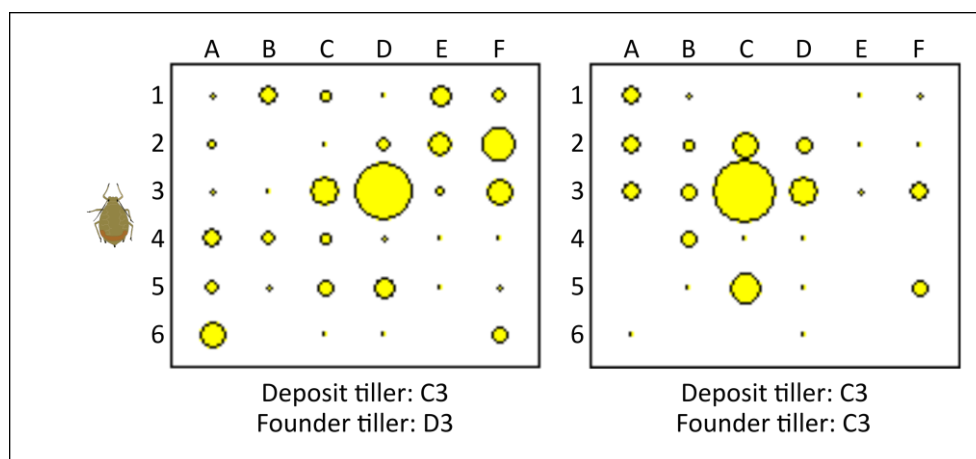
Annexe 1. Production céréalière annuelle en France (moyenne de 2020 à 2022). (Source : Agreste).

Céréale		Surface cultivée (unité=1000ha)	Rendement (q/ha)	Production (unité=1000 tonne)
Blé tendre	Hiver	4614	71	32591
	Printemps	26	64	168
Blé dur	Hiver	249	53	1330
	Printemps	16	52	84
Orge	Hiver	1220	63	7719
	Printemps	632	54	3357
Avoine	Hiver	52	42	222
	Printemps	48	41	197
Seigle		39	42	160
Triticale		312	49	1535
Maïs		1555	87	13452
Sorgho		84	50	420

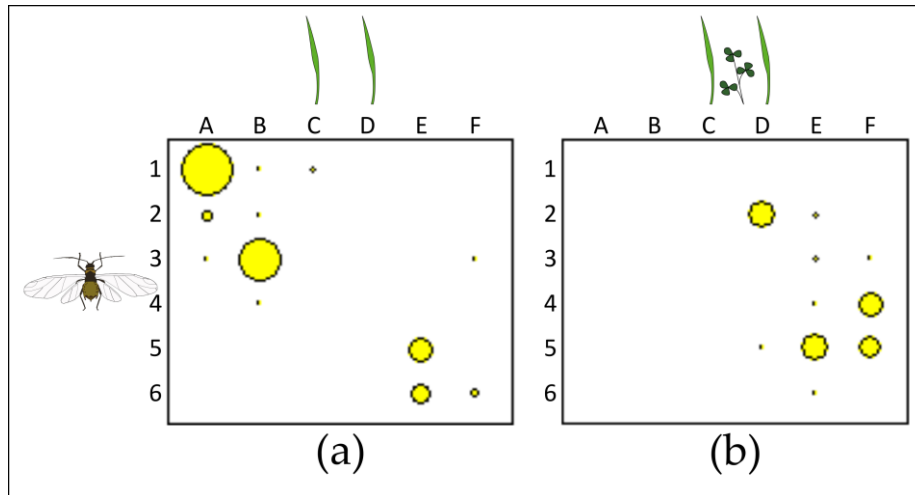
Annexe 2. Production céréalière annuelle dans la région Auvergne-Rhône-Alpes (moyenne de 2017 à 2021).

(Source : Agreste).

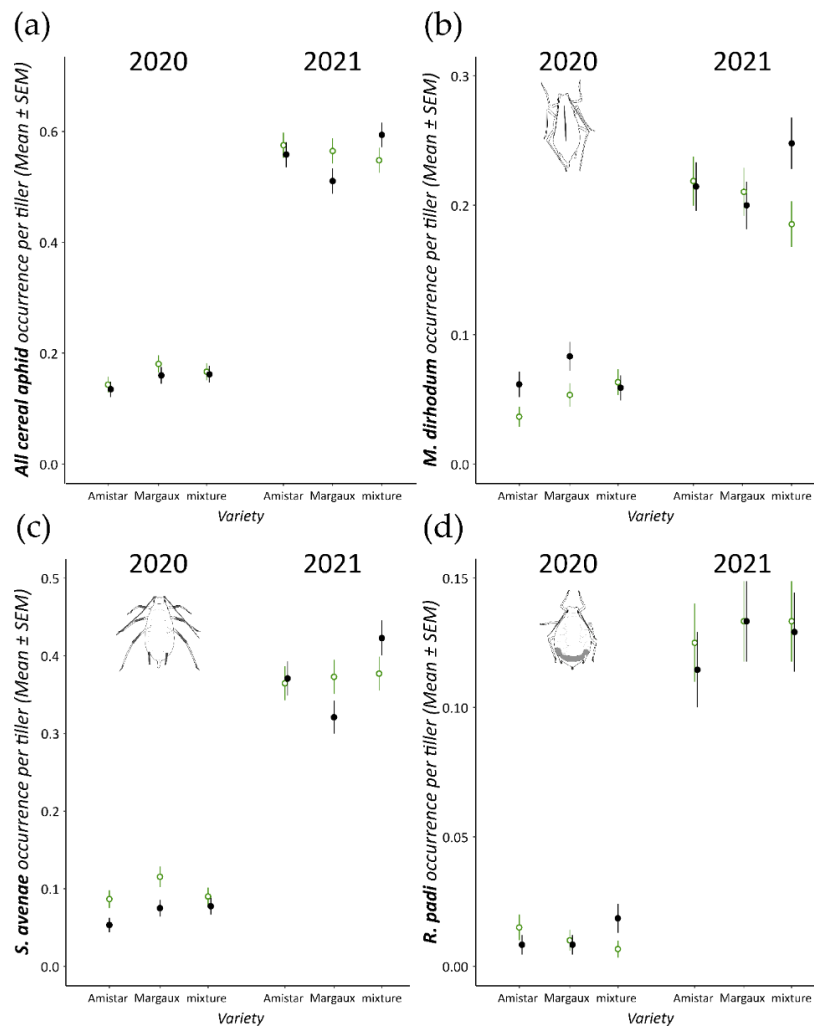
Céréale	Production (tonne)
Blé tendre	1 272 163
Maïs	1 210 979
Orge	368 285
Triticale	258 147
Blé dur	56 198
Sorgho	38 882
Seigle	35 544
Avoine	18 440
Total	3 258 638



Annexe 3. Examples of two arenas started with a wingless founder aphid after 13 days of experiment. (Data visualisation on R software). The arena is composed of 36 barley tillers. Each yellow point (●) represents the presence of at least one aphid (aphid occurrence=1) and the size of the point is proportional to the number of aphids per tiller. The larger point is the founder tiller. The deposit tiller is always the plant in position C3. The founder tiller may be different from the deposit tiller (early movement of the founder aphid). A single focus distribution of aphids is observed, where the number of aphids decreases as the distance to the founder tiller increases.



Annexe 4. Examples of two arenas started with an alate founder aphid after 13 days of experiment. (data visualisation on R software). The arena is composed of 36 barley tillers. Each yellow point (●) represents the presence of at least one aphid (aphid occurrence=1) and the size of the point is proportional to the number of aphids per tiller. **(a)** Barley alone treatment: a 2-focus distribution of aphids is observed **(b)** Barley-clover treatment: a one-focus distribution of aphids is observed.



Annexe 5. Occurrence of cereal aphids in spring regarding variety mixture or sole variety and barley intercropped or not with clover. Mean ± SEM per tiller of: **(a)**All cereal aphid species, **(b)** *M. dirhodum*, **(c)** *S. avenae*, **(d)** *R. padi*. Per treatment: n=600 tillers observed in 2020 and n=480 in 2021. Treatment with clover (○); Treatment without clover (●).

Title: Intra-field plant diversification and biological regulation of cereal aphids, vectors of the Barley yellow dwarf virus

Keywords: agroecology, pest control, intercropping, cultivar mixture, winter cereal, natural enemy, *Rhopalosiphum padi*, *Sitobion avenae*, BYDV

Abstract:

Development of crop protection practices towards greater sustainability, particularly in environmental and economic aspects, is a major challenge for the agroecological transition of cropping systems. Among crop pests, aphids are harmful insects that can cause significant yield losses, either directly or indirectly by transmitting viral diseases. This is the case for cereal aphids, which are the vectors of Barley yellow dwarf virus (BYDV). Aphids colonise and infect winter cereals as soon as they emerge in autumn and remain on the crop throughout the winter if temperatures are mild. The control methods to reduce aphid populations in autumn and winter are limited, especially in organic farming where the late sowing method is less effective with the greater frequency of mild winters. In spring, aphids can also cause significant damages if their populations are high.

This thesis focuses on a promising strategy to reduce pest populations: increasing intra-field plant diversity. Research carried out tested the potential of intra-specific diversification (mixture of winter barley varieties), inter-specific diversification (barley associated with a cover of different clover species) and the combination of the two types of diversification in order to: i) reduce aphid populations (alate, wingless) and yellow dwarf disease (YDD) prevalence in autumn-winter, and ii) reduce aphid populations and increase those of their natural enemies in spring.

Experiments have been implemented on ten farmers' organic fields, over the 2019-2020 and 2020-2021 growing seasons in the Rhône-Alpes region. On the experimental fields, populations of the different cereal aphid species, YDD prevalence, ground-dwelling predators and foliar enemies of aphids were monitored, as well as crop development and barley yield. A focused laboratory experiment on the effect of inter-specific diversification on the aphid *Rhopalosiphum padi* and the BYDV-PAV virus was conducted.

Intra-specific diversification did not reduce aphid populations. The effects of inter-specific diversification were variable depending on the period considered. In autumn-winter, the practice did not have a significant effect on aphids or on virus in farmers' fields, probably due to a limited development of the clover cover. Indeed, in the laboratory experiment, at the same sowing density but with a more developed cover, the clover allowed a reduction of *R. padi* populations per plant of almost 21% in abundance and 6% in occurrence and affected the spatial distribution of colonies produced by alate aphids. In spring, in farmers' fields, populations of some cereal aphid species were reduced in the presence of clover (*R. padi* and *Metopolophium dirhodum*) and the activity-density of some ground-dwelling arthropods was increased (ground beetles, lycosidae and rove beetles), while populations of foliar-dwelling natural enemies (parasitoid and hoverfly) were not. The two diversification types combined showed a slight significant effect in reducing the cereal aphid occurrence per plant in autumn-winter.

Among the types of intra-field plant diversification tested, inter-specific diversification appears to be the most promising for the cereal aphid regulation, despite a decrease in barley yield of around 15% caused by the crop association. Future work should aim to optimise the practice to increase the effects on the pest and its natural enemies while limiting the yield loss.

Titre : Diversification végétale intra-parcellaire et régulation biologique des pucerons des céréales, vecteurs du virus de la jaunisse nanisante de l'orge

Mots-clés : agroécologie, régulation des ravageurs, association culturale, mélange variétal, céréale d'hiver, ennemis naturels, *Rhopalosiphum padi*, *Sitobion avenae*, BYDV

Résumé :

L'évolution des pratiques de protection des cultures vers une plus grande durabilité notamment environnementale et économique est un enjeu majeur pour la transition agroécologique des systèmes de culture. Parmi les bioagresseurs, les pucerons sont des ravageurs qui peuvent entraîner des pertes de rendement importantes, par action directe ou indirecte en étant vecteurs de maladies virales. C'est le cas des pucerons des céréales, vecteurs du virus de la jaunisse nanisante de l'orge (Barley yellow dwarf virus - BYDV). Les pucerons colonisent et infectent les céréales d'hiver dès leur levée en automne et se maintiennent pendant l'hiver si les températures restent clémentes. Les leviers pour limiter le développement de leurs populations en automne-hiver sont peu nombreux, notamment en agriculture biologique où le levier du semis tardif voit son efficacité diminuer avec la plus grande fréquence d'hivers doux. Au printemps, les pucerons peuvent également provoquer des dégâts significatifs si leurs populations sont importantes.

Cette thèse s'est intéressée à un levier prometteur pour diminuer les populations de ravageurs : l'augmentation de la diversité végétale intra-parcellaire. Nos travaux ont testé le potentiel de la diversification intra-spécifique (mix de variétés d'orge d'hiver), inter-spécifique (orge associé à un couvert de différentes espèces de trèfle) et de la combinaison des deux types de diversification pour : i) diminuer les populations de pucerons (aillés, aptères) et la prévalence de la jaunisse nanisante de l'orge (JNO) en automne-hiver, et ii) diminuer les populations de pucerons et augmenter celles de leurs ennemis naturels au printemps.

Des expérimentations ont été mises en place sur dix parcelles d'agriculteurs, conduites en agriculture biologique sur les campagnes 2019-2020 et 2020-2021 en Rhône-Alpes. Sur ces parcelles, les populations des différentes espèces de pucerons des céréales, la prévalence de la JNO, les populations de prédateurs terrestres et d'ennemis foliaires des pucerons ont été suivies ainsi que le développement du couvert végétal et le rendement de l'orge. Une exploration plus spécifique de l'effet de la diversification inter-spécifique sur le puceron *Rhopalosiphum padi* et le virus BYDV-PAV a été réalisée en laboratoire.

La diversification intra-spécifique n'a pas permis de diminuer les populations de pucerons. Les effets de la diversification inter-spécifique, quant à eux, étaient variables selon la période considérée. En automne-hiver, la pratique n'a pas eu d'effet significatif sur les pucerons ou le virus en parcelles d'agriculteurs, probablement en lien avec un développement du couvert de trèfle limité. En effet, dans nos expériences en laboratoire, pour une densité de semis identique mais un couvert plus développé, le trèfle a permis une diminution des populations de *R. padi* par plante de près de 21% en abondance et de 6% en occurrence et a modifié la distribution spatiale des colonies issues des pucerons ailés. Au printemps, en parcelles d'agriculteurs, les populations de certaines espèces de pucerons des céréales ont été diminuées en présence de trèfle (*R. padi* et *Metopolophium dirhodum*) et l'activité-densité de certains arthropodes du sol a été augmentée (carabes, lycosidae et staphylins), au contraire des populations d'ennemis foliaires (parasitoïdes et syrphes). La combinaison des deux types de diversification a montré un léger effet significatif pour diminuer l'occurrence par plante des pucerons des céréales en automne-hiver.

Parmi les types de diversification végétale intra-parcellaire testés, la diversification inter-spécifique apparaît donc comme la plus prometteuse pour la régulation des pucerons des céréales, malgré une diminution du rendement de l'orge d'environ 15% causé par l'association. De futurs travaux devraient ainsi chercher à optimiser la pratique pour en augmenter les effets sur le ravageur ainsi que sur ses ennemis naturels tout en limitant la perte de rendement.