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**Dynamique des populations de méligèthes,  
Brassicogethes aeneus Fabr. (Coleoptera, Nitidulidae) et  
de son principal parasitoïde, Tersilochus heterocerus  
Thomson (Hymenoptera, Ichneumonidae) en fonction de  
l'hétérogénéité des paysages agricoles**

Amandine Juhel

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*Tersilochus heterocerus* Thomson (Hymenoptera,  
Ichneumonidae) en fonction de l'hétérogénéité des  
paysages agricoles.

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## Introduction générale





## **Introduction**

### **I. Contexte général**

Depuis plusieurs décennies, l'agriculture s'est intensifiée. Les rendements ont fortement augmenté de par une évolution des pratiques agricoles (Matson et al., 1997). L'utilisation d'intrants a elle aussi explosé. Les intrants correspondent à une large gamme de produits, tels que les fertilisants (engrais, amendements), les produits phytosanitaires (pesticides), ainsi qu'à des activateurs ou retardateurs de croissance. L'intensification agricole passe aussi par une simplification des pratiques, avec par exemple une généralisation du labour plutôt que d'autres méthodes de travail du sol. Cette intensification a aussi conduit à une simplification des paysages agricoles, qui s'est traduit par une suppression des espaces semi-naturels tels que les haies ou les friches.

Cette intensification agricole a de nombreuses conséquences et sur de nombreux niveaux. Ainsi, l'usage de tous ces produits phytosanitaires est largement remis en cause. Il conduit à la pollution des sols (Matson et al., 1997) et de l'eau (Berka et al., 2001). De plus, ces molécules sont faites pour attaquer une espèce cible mais en général elles ont un effet sur un spectre plus large et peuvent ainsi affecter d'autres espèces que celles pour lesquelles ils sont appliqués, et notamment l'homme (Margni et al., 2002). Leurs effets sur la santé humaine sont démontrés depuis quelques années, et provoquent des cancers, de l'asthme ou des problèmes neurologiques (Cimino et al., 2017, Kim et al., 2017). Les premiers concernés sont les agriculteurs mais des résidus de pesticides se retrouvent dans l'alimentation, ainsi l'ensemble de la population occidentale pourrait être impactée bien qu'à des niveaux d'exposition différents (Cimino et al., 2017, Kim et al., 2017). Les effets de ces produits sont aussi néfastes pour les écosystèmes (Margni et al., 2002), en affectant une espèce d'un écosystème, les pesticides impactent les autres espèces.

Les agroécosystèmes sont des écosystèmes particuliers, modifiés par l'homme. Or les écosystèmes apportent des services à l'Homme, dits services écosystémiques (Costanza et al., 2005). Ces services sont définis comme étant les bienfaits que l'Homme tire directement ou indirectement du fonctionnement des écosystèmes et des espèces en leur sein. Ces services ont été décrits et ce terme mis en avant pour défendre et protéger la biodiversité, c'est-à-dire la diversité des organismes, autant entre les espèces qu'au sein des espèces. Ainsi, dans les agroécosystèmes, de nombreux services existent comme des effets sur la structure du sol, la

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diversité génétique ou bien la purification de l'eau (Zhang et al., 2007). Ces services peuvent être classés en catégories, comme les services de régulation. Ces services sont des contrôles qu'effectue l'écosystème sur les apports qu'il peut fournir à l'Homme. Ils concernent par exemple la pollinisation, la purification de l'eau, la rétention du sol, ou bien la régulation naturelle des bioagresseurs (Zhang et al., 2007). L'intensification agricole diminue certains de ces services de régulation. Le déclin des pollinisateurs, tels que les abeilles (Henry et al., 2012) est le cas le plus connu mais la perte de biodiversité est beaucoup plus large (Geiger et al., 2010). Les espèces impliquées dans la régulation naturelle des bioagresseurs sont aussi largement affectés par les pesticides (Geiger et al., 2010, Theiling & Croft, 1988, Wilkinson et al., 1975).

Le contexte agricole actuel n'est plus durable. De nouvelles méthodes sont à développer pour continuer à produire et à avoir de bons rendements tout en limitant les impacts négatifs de l'agriculture sur l'environnement. Il est notamment important de limiter le recours des pesticides par l'agriculture et au contraire de favoriser le service de régulation des bioagresseurs.

### **II. La protection intégrée des cultures**

Ces difficultés appellent le développement d'alternatives à l'usage de produits phytosanitaires. Une des grandes stratégies mises en avant est la protection intégrée des cultures. Ce concept se base sur la lutte intégrée qui est un système de gestion des populations de bioagresseurs qui, dans un environnement donné et pour une espèce donnée, met en œuvre toutes les techniques appropriées pour maintenir les populations des espèces nuisibles à des niveaux inférieurs à ceux causant des dommages d'importance économique (Deguine et al., 2008). La protection intégrée des cultures se base sur cette lutte intégrée mais privilégie la mise en œuvre de régulations naturelles et prend en compte des impératifs écologiques et toxicologiques (Ferron et Deguine, 2005). Ce concept de protection intégrée doit bénéficier d'une vision très large du système pour prendre en compte l'ensemble des ressources et des processus de régulation naturelle existant au sein de l'écosystème et réduire les impacts sur l'environnement en assurant le développement d'une agriculture plus durable. Suivant ces principes, un ensemble de méthodes de lutte ont été développées à différentes échelles dans le temps et l'espace avec un accent fort sur la régulation naturelle des bioagresseurs (Eilenberg et al., 2001). Comme le soulignent Gurr et al., (2003) l'idée maitresse est de favoriser les capacités de régulation naturelle et de résilience de l'agro-système par deux modes d'action : bottom-up et top-down. L'approche "bottom-up" consiste à utiliser les caractéristiques de la plante hôte pour limiter le développement des ravageurs et leurs dégâts, et l'approche "top-down" consiste à stimuler les populations d'ennemis naturels. La première repose principalement sur l'impact des pratiques agricoles sur le fonctionnement du couvert et des bioagresseurs (travail du sol, choix variétal, nutrition azotée), permettant de stimuler les mécanismes de défense de la plante et les capacités de compensation, d'évitement. La deuxième repose majoritairement sur des pratiques agricoles à l'échelle du paysage et la gestion d'habitats et des aménagements paysagers favorisant le développement des auxiliaires des cultures

Une des méthodes de lutte existantes est la lutte génétique variétale, c'est-à-dire l'utilisation ou le développement de variétés résistantes par l'introduction de gènes de résistances d'origine bactérienne à des variétés sensibles ce qui permet de limiter les dégâts dus aux attaques de bioagresseurs (Smith et al., 2005, Flor et al., 1955). Une deuxième est la lutte physique, elle consiste à utiliser des moyens d'action physiques comme par exemple piéger les ravageurs pour limiter les dommages créés par les bioagresseurs ou à travailler le sol pour enfouir des mauvaises herbes des cocons d'insectes. Toutes ces méthodes relèvent d'un effet bottom up, via la modification du fonctionnement du couvert ou du milieu physique.

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Une troisième méthode et une des plus utilisées est la lutte culturale qui s'appuie sur la modification des pratiques agronomiques. Ces méthodes occupent une large gamme de possibilités comme décaler la date de semis (Sastawa et al., 2004), faire varier la densité de plantes (Ratnadass et al., 2012), augmenter les rotations (Trenbath, 1993) ou bien changer le type de travail du sol (Roger-Estrade et al., 2010). L'augmentation des rotations favoriserait aussi la présence des ennemis naturels des bioagresseurs (Khan et al., 1997 ; Rusch et al., 2013a). La lutte culturale passe aussi par l'utilisation de variétés ayant des cycles décalés par rapport aux cycles des ravageurs pour éviter une trop forte exposition (Cook et al., 2006). Ces différences de sensibilité et d'attractivité des différentes variétés de cultures ont amené à de nouvelles stratégies comme les cultures pièges (Hokkanen et al., 1991, Shelton & Badenes-Perez, 2006). Cette méthode consiste à mettre d'autres variétés ou d'autres plantes que la plante d'intérêt dans la parcelle pour dévier les ravageurs et pour qu'ils attaquent uniquement ces plantes pièges. Une autre méthode est la stratégie « push-pull » (Cook et al., 2007). Elle consiste à utiliser le comportement des insectes ravageurs en rendant répulsive la ressource (la culture, push) et en rendant attractive une autre source (pull) qui servirait de détournement.

Enfin une dernière lutte très utilisée est la lutte biologique. Elle consiste à utiliser des organismes vivants (insectes, bactéries, champignons, ...) pour limiter les populations de bioagresseurs. Cette dernière lutte se décompose en trois modalités. La lutte biologique par introduction, consiste à introduire un agent de lutte biologique qui réduirait les populations de bioagresseurs. C'est notamment le cas du parasitoïde *Tamarixia dryi* d'Afrique du Sud qui a été introduit à La Réunion dans les années 70 pour lutter contre *Trioza erythrae* (Van den Berg & Greenland, 2000). Cependant, ce type de lutte n'est pas sans danger et les espèces introduites peuvent devenir envahissantes (Howarth, 1991). Un deuxième type de lutte biologique est la lutte par augmentation. Elle consiste à augmenter la présence d'un ennemi naturel en l'élevant et en le relâchant dans la nature. Cette lutte est beaucoup utilisée contre la pyrale du maïs *Ostrinia nubilalis* avec des lâchers de *Trichogramma ostrinae* (Wright et al., 2001). Enfin, la lutte biologique vise à modifier l'environnement et les pratiques agricoles pour améliorer la survie et la régulation des ennemis naturels des bioagresseurs. Elle implique de laisser par exemple des espaces semi-naturels favorables à ces espèces. La lutte biologique par conservation doit prendre en compte des échelles spatio-temporelles plus larges que les autres méthodes de lutte qui s'applique généralement à la parcelle pour la saison en court (Tschardt et al., 2007).

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La protection intégrée des cultures amène donc un ensemble de méthodes de lutte qui s'appliquent en général à la parcelle, telles que le choix des variétés, des densités de semis ou du travail du sol. La lutte biologique par conservation, quant à elle, elle implique une échelle plus étendue : le paysage.

### **III. Le paysage : une échelle indispensable dans la lutte biologique par conservation**

Le contrôle biologique par conservation dans les agroécosystèmes nécessite de se placer dans une perspective plus large que l'échelle de la parcelle. En effet, la plupart des espèces d'arthropodes vivent dans différents habitats qui vont bien au-delà du niveau de la parcelle (Tschamntke et al., 2007). Malgré les nombreuses études et méta-analyses sur l'effet contrasté du paysage, c'est-à-dire qui améliore l'effet de certains ennemis naturels tout en améliorant aussi les abondances de certains ravageurs, l'effet du paysage et des habitats semi-naturels en particulier reste insuffisamment caractérisés (Bianchi et al., 2006, Holland et al., 2017).

D'une part, ces habitats sont des réservoirs d'ennemis naturels des ravageurs des cultures (Landis et al., 2000, Bianchi et al., 2006, Holland et al., 2017) qui les utilisent pour hiberner, se nourrir, ou en tant que refuges (Bianchi et al., 2006, Chaplin-Kramer et al., 2011). Par exemple, certaines araignées sont plus nombreuses lorsque le pourcentage d'habitats semi-naturels proches des cultures augmente (Schmidt & Tschamntke, 2005). D'autres prédateurs utilisent ces espaces pour hiberner (Pfiffner & Luka, 2000, Pywell et al., 2005). Or, le service apporté par ces ennemis naturels peut être majeur. Les populations de pucerons peuvent par exemple augmenter de plus de 100 % sans prédateurs ni parasitoïdes (Schmidt et al., 2003). Une mortalité de plus de 90% a pu être attribuée à des parasitoïdes, comme ceux des méligèthes (Büchi, 2002).

D'autre part, ces habitats sont également des lieux clés pour les ravageurs. En effet, certains ravageurs utilisent aussi des habitats semi-naturels, et donc des éléments du paysage autres que les parcelles, pour hiberner, se nourrir, ou en tant que refuges (Bianchi et al., 2006, Chaplin-Kramer et al., 2011).

Cette dépendance de divers habitats cultivés et non cultivés montre que comprendre les liens de dépendance spatiale entre les habitats semi-naturels et les cultures est important pour conserver la diversité des ennemis naturels tout en évitant de favoriser les ravageurs. Pour cela, comprendre l'utilisation des différents éléments paysagers ainsi que la dispersion des ennemis naturels et des ravageurs entre eux est un élément clé pour la gestion des ravageurs.

<p>Trouver des méthodes qui allient amélioration de la lutte biologique et diminution des dommages faits par le ravageur reste compliqué. Il est essentiel de mieux comprendre la biologie des ravageurs et de leurs ennemis naturels et notamment leur dispersion.</p>
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#### **IV. La dispersion : un élément clé difficile à estimer**

La dispersion peut être clé à plusieurs titres dans le bouclage du cycle de vie des insectes. Elle leur permet de se déplacer entre différents sites de nourrissage, mais aussi entre leurs sites d'hivernation et de reproduction (Stinner et al., 1983). La dispersion peut être estimée à l'aide de méthodes directes et indirectes.

Les méthodes directes concernent des techniques qui permettent d'observer la dispersion, les mouvements qu'effectuent les individus. Ce sont par exemples les techniques de capture marquage recapture. On capture un individu à un lieu A, on le marque avec un élément radioactif ou de la peinture par exemple ou en enlevant des poils pour un mammifère, on le relâche et on le recapture en un lieu B. La distance entre ces lieux peut ainsi être mesurée. Cette méthode a été utilisée pour mesurer la dispersion de moustiques par exemple (Muir & Kay, 1998). Cependant, cette technique est difficilement applicable sur des insectes ravageurs dont les populations sont en général très fortes. La plus utilisée et la plus facile à mettre en place a été l'utilisation d'éléments radioactifs mais cette méthode est très polémique. Du radiopistage peut aussi être utilisé, en plaçant un GPS sur l'individu suivit. Du radiotraquage a été réalisé sur des toucans (Kay et al., 2010). Sur certains insectes assez gros (quelques centimètres), ces méthodes ont été aussi appliquées (Vinatier et al., 2010). Mais la plupart des ravageurs des grandes cultures sont plus souvent plus petits qu'un centimètre.

D'autres méthodes moins directes ont été développées à l'aide de la génétique. Ainsi, des méthodes d'assignation de parentés permettent, à l'aide de marqueurs moléculaires de trouver des liens de parentés entre individus tels que parents-enfants et de mesurer la distance qui se trouve entre ces individus (Jones & Arden, 2003). Ces méthodes ont par exemple été utilisées pour des ravageurs comme le carpocapse du pommier (Franck et al., 2011).

Enfin des méthodes indirectes existent, ce sont des méthodes qui observent les patrons génétiques engendrés par la dispersion. Ces méthodes estiment les flux de gènes entre populations (Broquet & Petit, 2009). A partir de la structure génétique des populations, c'est-à-dire des liens entre différentes populations d'une même espèce estimés à l'aide de marqueurs moléculaires, la dispersion peut être estimée. Cependant, les populations de ravageurs des grandes cultures sont potentiellement si nombreuses que le potentiel de ces outils génétiques est encore incertain. D'autres méthodes indirectes se basent sur des analyses d'abondances avec des kernels de dispersion et qui prennent en compte les différentes sources possibles de provenance des individus (Nathan et al., 2012).



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De nombreuses méthodes directes et indirectes sont utilisées pour mesurer la dispersion. Cependant, dans le cas d'insectes, les méthodes générales de capture marquage recapture ou de radiopistage ne peuvent être mises en place ou difficilement. Des méthodes génétiques, quant à elles semblent être adaptées.

Nous proposons ici d'utiliser conjointement des méthodes directes et indirectes pour mesurer la dispersion et la dynamique des populations d'un ravageur du colza, le méligèthe et de son parasitoïde principal en fonction de l'hétérogénéité des paysages agricoles.

## V. Système étudié et bilan des connaissances actuelles

### I. *Le colza d'hiver*

Le colza d'hiver, *Brassica napus*, est une culture qui a été en expansion en France ces 30 dernières années et qui a maintenant tendance à stagner (Hokkanen et al., 2000) (Figure 1).

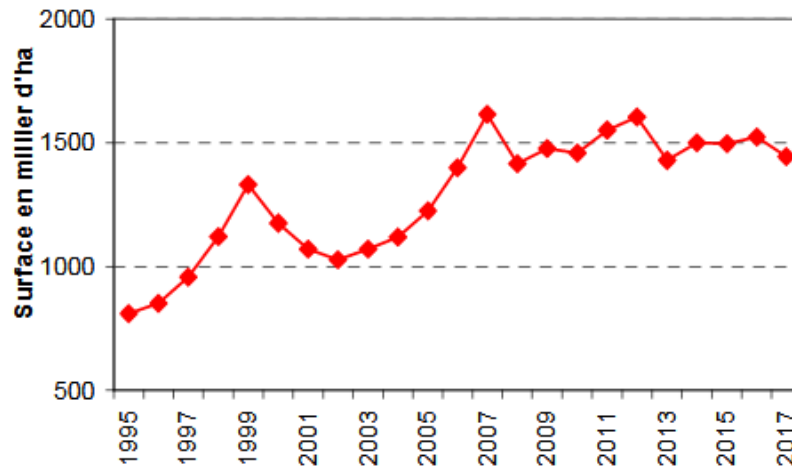


Figure 1 : Evolution des surfaces cultivées en colza en France de 1995 à 2017. Source : Agreste conjonctures, 2016 N°10/10.

Cette culture est emblématique de la simplification des rotations, elle est en effet intégrée dans une rotation colza, blé, orge. Elle a de nombreux atouts, d'abord économiques avec la valorisation en huile pour la consommation humaine, tourteaux pour l'élevage mais aussi en tant que biocarburant. De plus, le colza possède de bonnes capacités d'absorption de l'azote ce qui limite la lixiviation du sol (lessivage du sol par les pluies) (Dejoux et al., 2003). Enfin la bonne fixation du pied du colza dans le sol améliore la qualité du sol et aide au rendement de la céréale, habituellement du blé, qui suit le colza dans la rotation. Cependant, comme toute culture, le colza a aussi des défauts, le principal étant que, du fait de son retour très rapide dans la rotation, il est soumis à de nombreux bioagresseurs tout au long de son développement. En automne, au moment de son implantation et de ses premiers développements, le colza est attaqué par des limaces, des insectes variés (altises, mouche du chou, charançon du bourgeon terminal, etc) (Alford et al., 2003) mais aussi des maladies (phoma). Au printemps et jusqu'au début de l'été, il est attaqué par d'autres insectes (charançon de la tige, charançon des siliques, méligèthes) (Alford et al., 2003) et maladies (oïdium, sclérotinia). En France, ce nombre important de bioagresseurs suscite une utilisation importante de produits phytosanitaires, la deuxième en grande culture après la pomme de terre (Agreste, 2016). En effet, son indice de fréquence de traitement, qui est la mesure des traitements phytosanitaires utilisés sur la culture

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en fonction de la surface, était de 6.51 contre 18.92 pour les pommes de terre et 4.93 pour le blé en 2014. Des alternatives à ces pesticides sont donc attendues pour diminuer leur usage.

Le colza est une culture soumise à de nombreux bioagresseurs. De ce fait elle est l'une des grandes cultures les plus traitées. Réduire les dégâts occasionnés par ces bioagresseurs est un enjeu majeur.

### **II. Méthodes de lutte contre les ravageurs du colza**

De nombreuses méthodes de lutte face aux ravageurs du colza existent mais à l'heure actuelle, la lutte chimique est principalement utilisée. Les méthodes les plus utilisées ou discutées sont présentées ci-dessous.

#### *Lutte chimique et résistance*

En 2008, de 0 à 5 traitements étaient effectués sur le colza dans la plupart des pays européens, principalement contre les méligèthes par exemple (Richardson et al., 2008). Les principales molécules utilisées étaient des pyréthriinoïdes, des composés organophosphorés et des composés organochlorés (Richardson et al., 2008). En presque 10 ans, la situation n'a pas changé, les traitements contre les ravageurs du colza sont toujours aussi nombreux. Cette forte fréquence des traitements a conduit à l'apparition et au développement de résistances chez les méligèthes depuis les années 2000 (Hansen, 2003), ou bien chez les altises (Zimmer et al., 2014). De nombreuses études ont montré que ces résistances aux pyrétroïdes se sont développées et répandues dans toute l'Europe pour les méligèthes (Veromann et al., 2011, Slater et al., 2011).

#### *Utilisation de plantes pièges*

Une partie des stratégies évoquées plus haut pour remplacer les pesticides a été testée sur les ravageurs du colza (Cook et al., 2008). Ainsi, l'utilisation de plantes pièges a beaucoup été testée, notamment avec d'autres espèces de brassicacées et principalement la navette (*Brassica napus*) (Cook et al., 2006). Cette espèce fleurit quelques jours avant le colza et permet ainsi de détourner les méligèthes et les altises du colza (Cook et al., 2008, Barari et al., 2005). Cette attraction des méligèthes par la navette est aussi utilisée dans les stratégies de Push-Pull (Cook et al., 2007). En effet en mettant de la navette autour de parcelles dans lesquelles est cultivée une variété de colza répulsive, on peut divertir les méligèthes de la culture (Cook et al., 2007). Les différentes variétés de colza sont beaucoup étudiées pour répondre à ce type de stratégies ainsi que pour améliorer les connaissances de leurs effets sur la survie et le comportement des méligèthes (Hervé et al., 2004).

### **III. Le méligèthe du colza**

Comme indiqué ci-dessus, le méligèthe, *Brassicogethes aeneus* (anciennement appelé *Meligethes aeneus* F.) fait partie des insectes causant des dommages importants aux cultures de

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colza. Ce coléoptère univoltin, c'est-à-dire qui ne fait qu'une génération par an, se nourrit principalement de pollen aux stades adulte et larvaires et notamment du pollen de brassicacées (Cook et al., 2004) ce qui entraîne des pertes de rendement (Williams & Free, 1978, Free & Williams, 1978a) (Figure 3f). Les dégâts causés par les méligèthes peuvent conduire à des pertes de rendement de 30 à 40 % sur le colza d'hiver à l'échelle de la parcelle (Nilsson, 1994). Cependant, le colza possède de fortes capacités de compensation : si les attaques se font tôt dans le cycle, la plante peut faire de nouvelles ramifications et combler le déficit de boutons sur les ramifications principales (Williams & Free, 1979). Néanmoins le poids des graines de ces plantes sera plus faible, surtout en cas de carence en azote (Rusch et al., 2013b, Williams & Free, 1979).

Le cycle de vie du méligèthe présente des phases bien distinctes temporellement et spatialement, représentant autant de points de régulation potentiels (Figure 2, détaillé en Figure 3). *Brassicogethes aeneus* hiverne en tant qu'adulte dans la litière de feuille, essentiellement dans les éléments semi-naturels tels que les bois ou les haies (Rusch et al., 2011). Lorsque la température atteint 10 °C (Nilsson, 1988a), les adultes peuvent sortir de diapause pour se nourrir du pollen de diverses plantes (Free & Williams 1978b) (Figure 3a). Les femelles deviennent matures à cet instant du cycle (Free & Williams, 1978a) et, lorsque les températures atteignent 15°C, cherchent leur plantes hôtes, c'est-à-dire le colza (Williams, 2010, Cook et al., 2006, (Ekbom & Borg 1996). Les méligèthes peuvent parcourir 200 à 300 m en 2 heures lors de cette phase et jusqu'à 12 000 m en 2 jours selon une expérience de capture-marquage-recapture (Taimr et al., 1967). Le rôle des prairies, des bords de chemins et des jachères n'est pas connu à ce moment du cycle.

Une fois les parcelles de colza atteintes, les deux sexes se nourrissent du pollen des fleurs de colza et s'accouplent dans ces parcelles (Figure 3b,c). Les femelles creusent de petits orifices dans les bourgeons de colza les plus gros, c'est-à-dire faisant 2 à 3 mm et y déposent leurs œufs (Free & Williams 1978b, Nilsson, 1988b). Chaque femelle dépose en moyenne entre 100 et 200 œufs au cours de sa vie (Free & Williams 1978b). L'alimentation des adultes provoque l'avortement des boutons et ainsi des pertes de siliques (Williams & Free, 1978, Free & Williams, 1978a) (Figure 3f). L'oviposition amène aussi une perte de rendement mais elle est négligeable comparé à la perte liée à l'alimentation des adultes. Ainsi, ce ne sont pas les larves qui font le plus de dégâts, excepté lors de fortes infestations.

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Ensuite, l'œuf éclot dans le bouton et le premier stade larvaire se développe en se nourrissant du contenu du bouton. Le second stade larvaire se nourrit des fleurs se trouvant à proximité (Cook et al., 2004) (Figure 3d). Après ces deux stades larvaires, deux semaines se sont écoulées, la larve tombe au sol et se nymphose dans les premiers centimètres du sol (Williams, 2010). Après quelques semaines dans le sol et avant la récolte du colza, l'adulte de la nouvelle génération émerge, mi-juin en France (Williams & Free 1978). Ces nouveaux adultes se dispersent en se nourrissant du pollen de diverses plantes qu'ils trouvent (Ouvrard et al., 2016) avant d'aller dans leurs espaces d'hivernation (Figure 3 e). Lors de cette phase de dispersion, une seule expérience de capture-marquage-recapture a montré que les mélégièthes pouvaient parcourir de 1 000 à 3 000 m en une journée lorsqu'ils émergent des parcelles de colza en été (Stechmann & Schütte, 1976).

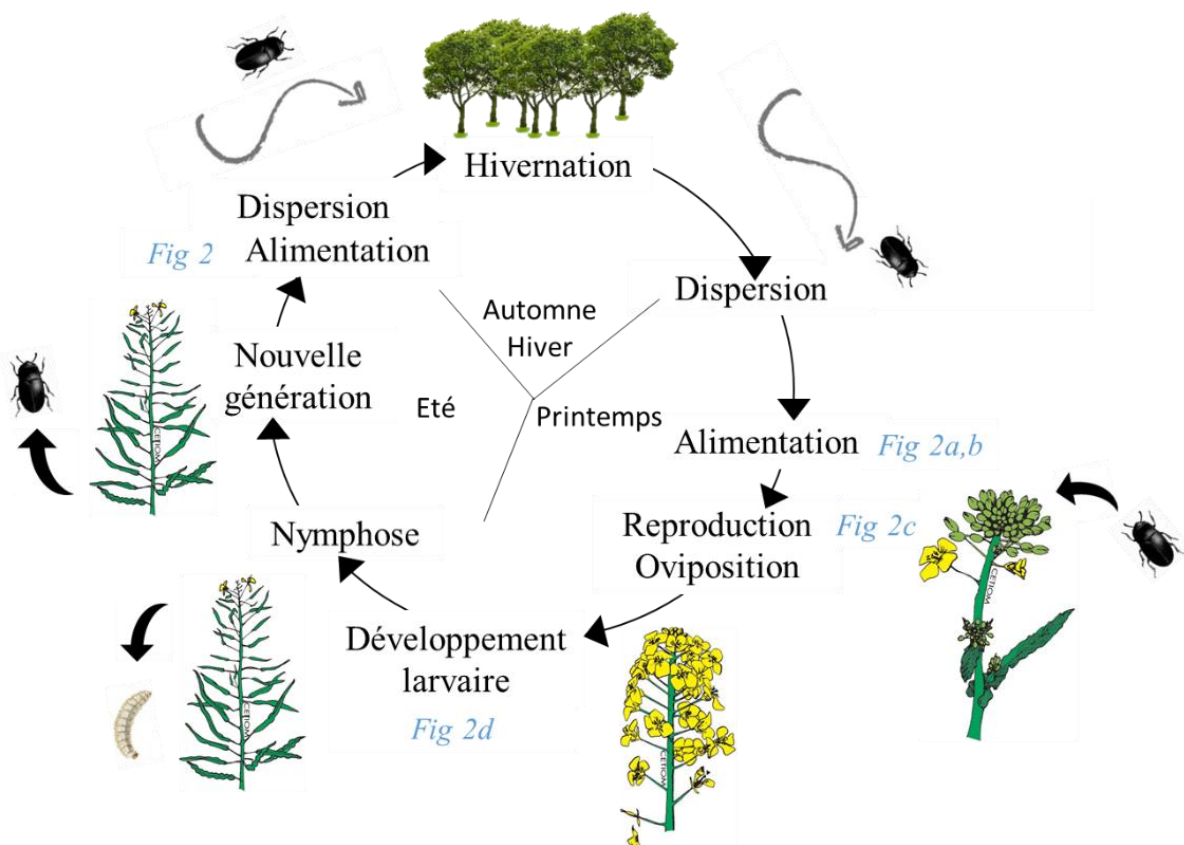


Figure 2. : Résumé du cycle de vie de *Brassicogethes aeneus* (Fabricius). En bleu : référence à des photos de la figure 2. (Images colza : Terre Inovia)

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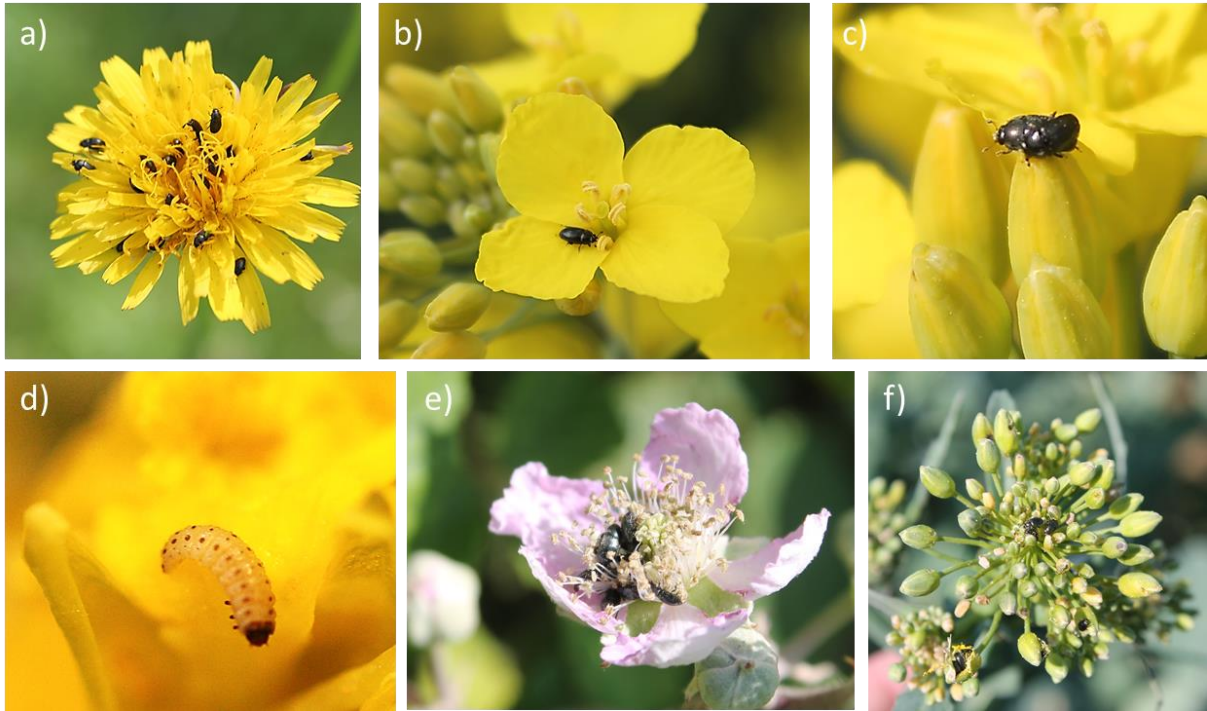


Figure 3 : Méligèthes à certains moments de leur cycle de vie. a) sur un pissenlit au printemps, b) méligèthes s'alimentant du pollen d'une fleur de colza, c) reproduction de méligèthes, d) larve de méligèthes stade 2 e) méligèthes s'alimentant sur des fleurs de familles variées en été, ici sur un églantier, f) dégâts de méligèthes (perte de boutons floraux) sur le colza. Photos : A.S. Juhel

Le méligèthes est un ravageur qui peut causer des dégâts majeurs sur le colza, et en impacter fortement le rendement. Son cycle de vie est complexe et implique des mouvements importants dans le paysage agricole entre ses différents habitats.

### **IV. Les ennemis naturels des méligèthes**

Les méligèthes, comme la plupart des ravageurs, possèdent de nombreux ennemis naturels (Williams, 2010, Riggi et al., 2017). Une part importante de la mortalité des méligèthes est due aux prédateurs tels que les carabes, les staphylyns ou les nématodes (Schlein & Büchs, 2004, Nielsen & Philipsen, 2005). L'impact des prédateurs peut être très important au moment de la nymphose, en avril-mai bien que les prédateurs tels que les carabes sont moins présents (Hokkanen, 2004).

Les populations de méligèthes sont aussi affectées par des entomopathogènes tels que *Beauveria bassiana* et *Metarhizium anisopliae* (Butt et al., 1994). Ces espèces ont un effet négatif sur les méligèthes lors de l'hivernation (Hokkanen, 1993).

Une grande part de la régulation naturelle des méligèthes est faite par les parasitoïdes (Büchi, 2002). En effet, on peut trouver jusqu'à plus de 90 % de larves de méligèthes parasitées dans certaines régions (Büchi, 2002). Mais ce taux est très variable et peut aussi être de 0 % (Büchi et al., 2002, Rusch et al., 2012). Cependant les raisons de ces variations sont mal connues. Trois espèces d'hyménoptères univoltins de la famille des ichneumonidés sont majoritaires en Europe (Jönsson et al., 2004 ; Veroman et al., 2006) : *Phradis interstitialis* (Thomson, 1889), *Phradis morionellus* (Holmgren, 1860) et *Tersilochus heterocerus* (Thomson, 1889). Cette dernière est prédominante et est la plus étudiée (Büchi et al., 2002). Cette dernière espèce étant la plus importante, c'est elle qui va être étudiée dans la suite de ce travail.

Le cycle de vie de cette espèce est décrit dans la Figure 4. Après s'être reproduites avec les mâles, les femelles parasitoïdes déposent leurs œufs dans les larves de méligèthes, à l'intérieur des boutons et des fleurs de colza. La larve de méligèthes se développe normalement et tombe au sol pour se nymphoser. L'œuf de parasitoïde éclot alors et se développe en quelques semaines dans la larve de méligèthes. Ce nouvel adulte parasitoïde reste dans le sol jusqu'au printemps suivant (Williams, 2006).



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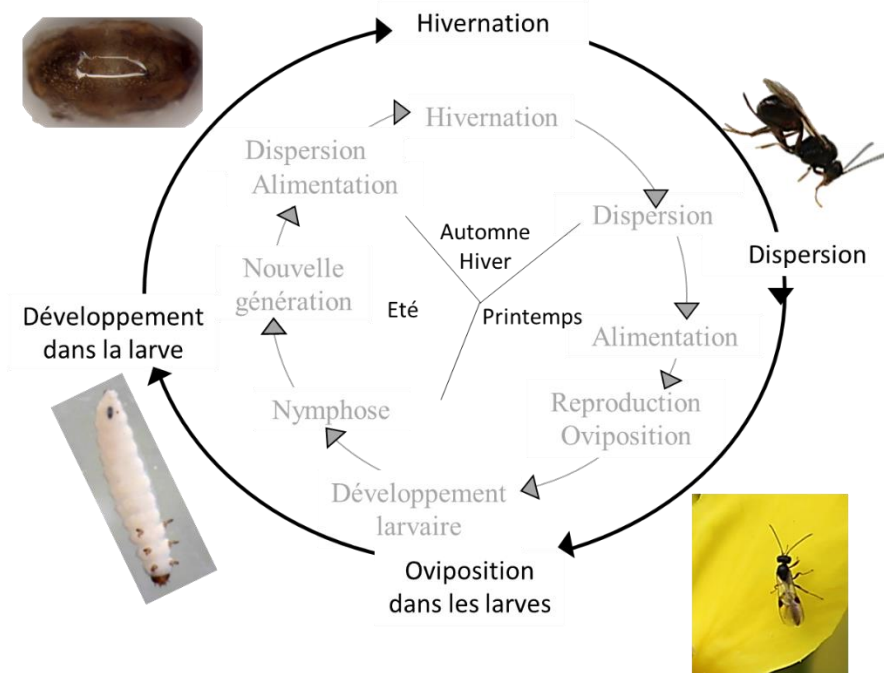


Figure 4 : Cycle de vie de *Tersilochus heterocerus*, parasitoïde principal des méligèthes.

Le parasitisme des méligèthes par cette espèce est connu pour être influencé par le paysage. Plus le paysage est complexe (Thies & Tschardtke, 1999) et possède des bois et des prairies (Rusch et al., 2012) et plus le taux de parasitisme est élevé. Par ailleurs, le travail du sol semble impacter négativement la survie des nymphes de parasitoïdes (Williams, 2006). Le fait de travailler la terre enfouit profondément les cocons de parasitoïdes qui en meurent (Nilsson, 2003). Cependant, une autre étude a montré que le travail du sol n'avait qu'un effet limité sur la survie des cocons (Hanson et al., 2015). Une autre pratique culturale a un effet négatif sur les parasitoïdes : l'usage de pesticides dans le colza. Les parasitoïdes y sont très vulnérables à leur émergence en mai-juin (Williams, 2006 ; Hanson et al., 2015).

*T. heterocerus* a tendance à émerger de fin avril à début juin (Williams, 2006, Berger et al., 2015, Berger et al., 2015). Les femelles oviposent dans les larves de méligèthes présentes sur les fleurs de colza déjà ouvertes (Berger et al., 2015). De plus *T. heterocerus* semble préférer les larves de méligèthes de stade aux larves de stade 2 (Berger et al., 2015). La nutrition de ces parasitoïdes a peu été étudiée mais il semblerait qu'ils se nourrissent de pollen et de nectar de fleurs, possiblement de fleurs de colza (Rusch et al., 2013c). Ce phénomène est corroboré par le fait qu'elles sont attirées par des stimuli visuels et olfactifs comme l'odeur des plantes (Berger et al., 2015). Elles sont notamment attirées par les composés volatiles émis par le colza lorsqu'il est attaqué par des ravageurs (Jönsson et al., 2005 ; Jönsson & Anderson, 2007). Ces attractions pour les odeurs et les couleurs du colza font que les parasitoïdes, tout comme les

méligèthes, remontent le vent pour trouver leurs hôtes (Williams et al., 2007). Cependant, leurs capacités de dispersion restent incertaines.

### *V. Le rôle du paysage dans la dispersion et la survie de ces insectes*

Pour les deux espèces, la dispersion a été peu étudiée. Nous avons montré que quelques études avaient mesuré la dispersion des méligèthes à l'aide de capture marquage, recapture mais à notre connaissance rien n'est connu sur la dispersion des parasitoïdes.

Or, ces dispersions sont capitales pour la survie à la fois des insectes ravageurs et de leurs ennemis naturels. Les distances entre patches d'habitats pourraient être déterminantes dans leur capacité à réaliser le cycle de vie complet. Cette information est aussi capitale pour savoir comment et à quelle échelle du paysage intervenir que ce soit pour piéger les ravageurs (lutte culturale - bottom up), ou pour favoriser les ennemis naturels (lutte biologique par conservation - top down).

Un travail de modélisation est venu confirmer l'importance à la fois de la dispersion et du rôle exact des habitats dans la conception de scénarios paysagers : un modèle spatialement explicite permettant de tester différents scénarios de pratiques (pesticides, travail du sol, rotation) dans des matrices paysagères contrastées a été conçu sur la base de connaissances partielles des interactions méligèthes-parasitoïdes-paysage (Vinatier et al., 2012). L'analyse de sensibilité a montré que les sorties du modèle, c'est-à-dire les dommages occasionnés par les méligèthes, dépendaient fortement des paramètres démographiques renseignée pour chacune des espèces (Vinatier et al., 2013). Au niveau des méligèthes, ces paramètres étaient liés à la distance de dispersion des méligèthes après l'hivernation et à leur survie durant cette dispersion. Des paramètres démographiques étaient aussi importants comme la survie des individus pendant l'hivernation et leur densité. De nombreux paramètres sur la démographie des parasitoïdes étaient aussi très influents sur les dommages occasionnés par les méligèthes, comme la durée de leur dispersion pour leur alimentation et pour leur reproduction. Cette analyse de sensibilité montre à quel point les paramètres démographiques de ces espèces sont essentiels pour caractériser et mieux contrôler les populations de méligèthes à l'aide des populations de parasitoïdes.

Les connaissances sur la biologie et l'écologie des méligèthes et de leurs parasitoïdes sont assez bien connues. Cependant leurs capacités de dispersion restent peu étudiées et incertaines.

## **VI. Questions de recherche, démarche générale du travail et organisation de la thèse**

La revue de la littérature sur la biologie des méligèthes et de leur parasitoïde a montré que des informations sur la dispersion de ces espèces et la dynamique de leur populations restent incertaines. **L'objectif général de ce travail est de comprendre les dynamiques spatiales et temporelles des populations de méligèthes et de leur parasitoïde principal *Tersilochus heterocerus* dans des paysages hétérogènes.**

Partant de l'échelle continentale, nous nous sommes intéressés au niveau de différenciation génétique des populations de méligèthes européennes. Compte tenu de la rapidité de propagation de la résistance aux insecticides, nous supposons d'important flux de gènes entre les populations de méligèthes européennes. Nous avons développé des marqueurs microsatellites pour mesurer la structure génétique de ces populations de méligèthes. Ce travail fait l'objet du premier chapitre de la thèse et correspond à une publication soumise en août 2017 dans *Pest Management Science* et intitulée « Limited genetic structure of *Brassicogethes aeneus* populations in France and in Europe ».

Nous nous sommes ensuite intéressés à la dynamique des populations de méligèthes dans une zone plus restreinte et cherché à estimer la taille efficace de ces populations. Nous nous sommes questionné sur l'évolution de cette taille efficace au fil d'une génération. Nous avons fait l'hypothèse que nous allions trouver une taille efficace plus faible, dans les parcelles de colza au printemps que l'année suivante, toujours dans les parcelles de colza mais après leurs deux phases de dispersion. Nous avons utilisé les marqueurs microsatellites que nous avons développés pour réaliser une analyse de parentés et ainsi estimer ces tailles efficaces de populations. Ce travail fait l'objet du chapitre deux et d'un article pas encore soumis intitulé « Sibship assignments and effective population size measure of an oilseed rape pest ».

Alors que l'on sait que lors de la phase de dispersion du printemps les méligèthes quittent les bois pour accéder aux parcelles, le chapitre trois s'interroge sur la distance qu'ils peuvent parcourir pour rejoindre l'habitat utile à leur reproduction, ainsi que leur progression au sein

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d'une parcelle. Nous avons utilisé de la modélisation et des mesures d'abondance pour mesurer cette distance. Ce travail fait l'objet du troisième chapitre et d'une publication acceptée dans le journal PlosOne intitulée « Characterization of the pollen beetle, *Brassicogethes aeneus*, dispersal from woodlands to winter oilseed rape fields. ».

Lors des deux phases de dispersion, les habitats que les méligèthes peuvent utiliser en tant que ressource, autre que le colza, restent mal connus. Ici nous nous sommes donc demandé quels habitats ils pouvaient utiliser. Nous avons fait l'hypothèse que les habitats semi-naturels tels que les prairies pouvaient être des habitats utilisés lors des deux phases de dispersion et que les parcelles cultivées par une autre culture que du colza pouvaient aussi être une ressource en été. Nous avons échantillonné des méligèthes dans ces différents habitats au printemps et en été pour répondre à cette question. Ce travail fait l'objet du chapitre quatre et est liée à un article non soumis intitulé « Wild flowers, important pollen source for an oilseed rape pest ».

Enfin, nous avons montré que la régulation biologique des méligèthes par les parasitoïdes passait par une meilleure connaissance de la biologie des parasitoïdes et de leurs déplacements. Afin de mieux comprendre la dynamique des populations de parasitoïdes à l'échelle Européenne, nous nous sommes demandés si les populations de différents pays européens étaient liées et avaient la même structure génétique. Nous avons développé des marqueurs moléculaires pour comparer des populations européennes de *T. heterocerus* entre elles et pour mesurer des distances entre parents et descendants. Ceci fait l'objet du chapitre six et s'intitule « Using microsatellite markers to improve knowledge of a crop pest parasitoid, *Tersilochus heterocerus*. »

Nous nous sommes finalement intéressés à l'importance relative du paysage et de la présence de méligèthes pour expliquer la présence de parasitoïdes sur des parcelles de colza. En particulier nous étudions si l'effet du paysage est confirmé même lorsque les abondances de méligèthes sont prises en compte. Nous avons échantillonné des parasitoïdes dans différentes parcelles et réalisé des modèles statistiques sur ces données. Ceci fait l'objet du chapitre six et s'intitule « Impact of landscape elements on a parasitoid abundance obfuscated by its host abundance ».

Nous abordons ces questions en 6 chapitres présentés sous la forme d'articles. Ils sont précédés d'un matériel et méthode synthétique revenant sur les grandes lignes des dispositifs et sur les

choix méthodologiques. Nous revenons enfin sur l'ensemble des résultats obtenus dans la discussion générale.

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## Introduction

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## Matériel et Méthodes

Dans cette partie sont présentés la zone d'étude, la sélection de parcelles, la cartographie de cette zone, les principales mesures effectuées et le paramétrage des logiciels de génétique. Le détail des échantillonnages et des expérimentations est fourni plus précisément dans les différents chapitres de cette étude.

### I. Sites d'étude

Pour tester les différentes hypothèses de notre travail et répondre à nos objectifs, nous avons dans un premier temps choisi une zone d'étude en Normandie (coordonnées du centre de la zone : 48°55'41.8"N, 1°19'26.7"E) (Figure 1). Cette zone présente plusieurs caractéristiques intéressantes pour nos objectifs (i) les attaques de méligèthes sont encore fréquentes même si elles sont variables et parfois faibles, alors que dans beaucoup de zones proches de l'ouest parisien de très faibles attaques avaient été répertoriées. (ii) les surfaces de colza et d'habitats semi-naturels varient beaucoup sur une assez courte distance pouvant ainsi avoir dans un rayon de 15 km soit des zones boisées, en vallée, bocagères et des paysages plus openfield avec comme seuls habitats semi-naturels de petits bosquets d'arbres. La zone d'étude choisie présente un gradient de complexité partant de la vallée de l'Eure, avec beaucoup d'habitats semi-naturels tels que des bois, des haies ou des prairies, allant jusqu'au plateau de St André, comportant, au contraire, peu d'habitats semi-naturels.

Ainsi trois zones de cette région ont été utilisées selon la question et le chapitre développé (Figure 5) :

La zone A a servi à échantillonner des méligèthes pour le calcul des distances de dispersion du chapitre 2. Elle a aussi servi à échantillonner des méligèthes pour l'étude de structure génétique en Europe. Elle a enfin servi de point de départ pour l'échantillonnage des méligèthes pour l'étude d'apparement.

La zone B a servi à échantillonner des méligèthes et des parasitoïdes pour les études de génétique (chapitre 5) et pour regarder l'effet du paysage sur les parasitoïdes (chapitre 6).

Enfin la zone C a servi à suivre une génération de sa naissance à sa reproduction (chapitre 3) et à étudier dans quels autres habitats que les parcelles de colza vont les méligèthes au moment de leur dispersion (chapitre 4).

## Matériel et méthodes

En parallèle de cette zone d'étude dans l'Eure, nous avons échantillonné des parcelles de colza dans différents pays européens pour les deux chapitres sur la structure des populations de melligèthes (chapitre 1, Figure 2) et de parasitoïdes (chapitre 5) (Figure 3). Ainsi, pour les melligèthes, nous avons sélectionné quelques-uns des points d'échantillonnage de l'Eure de 2016. Nous avons échantillonné 7 autres parcelles en France et 5 autres dans d'autres pays Européens (Figure 6).

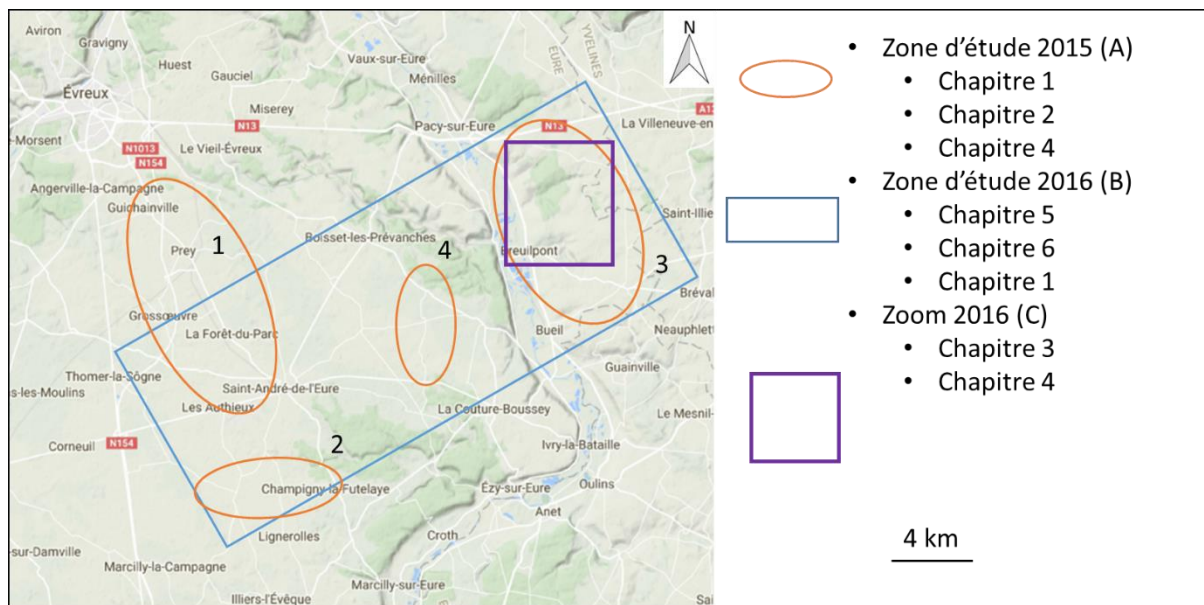


Figure 5 : Zone d'étude de la thèse avec les principales zones d'échantillonnage en fonction des années et le lien avec les différentes parties. Les chiffres correspondent aux 4 sites oranges de la zone A

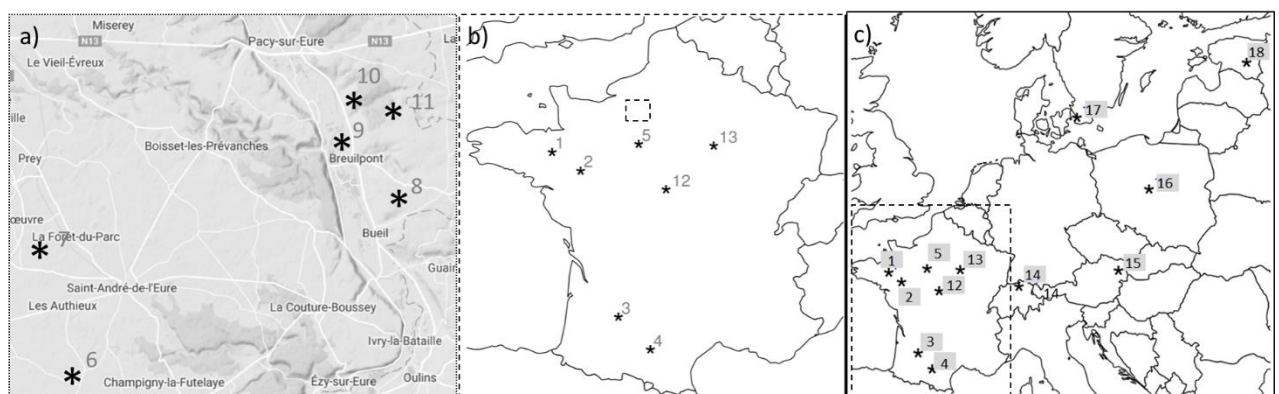


Figure 6 : Localisation des parcelles de colza dans a) l'Eure, b) la France, c) l'Europe.

Pour les parasitoïdes, nous avons échantillonné des melligèthes dans 5 parcelles de différents pays Européens et dans une centaine de parcelles dans la zone B de l'Eure en 2016 (Figure 7).

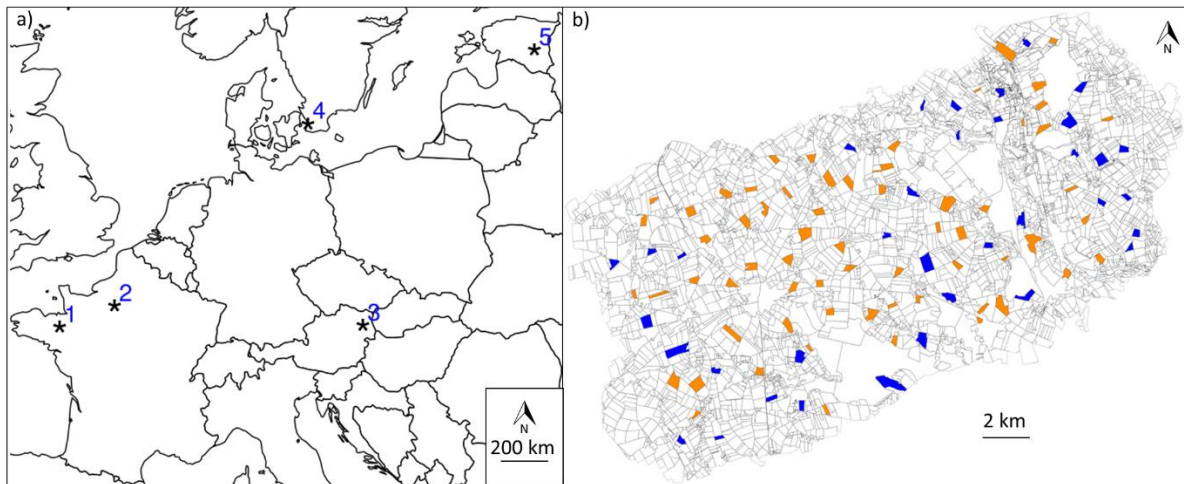


Figure 7 : Parcelles de colza échantillonnées pour les études de génétique a) en Europe, b) dans la zone B de l'Eure. En orange : parcelles échantillonnées avec des parasitoïdes, en bleu, parcelles sans parasitoïdes.

## II. Cartographie

Pour pouvoir mener des analyses sur le paysage, et mettre en relation les surfaces cultivées et non cultivées avec les abondances de méligèthes et/ou de parasitoïdes, nous avons cartographié la zone d'étude, c'est-à-dire la zone A en 2015 et la zone B en 2016. Nous avons utilisé Google MyMaps pour définir le contour de toutes les composantes de la zone, que ce soient les parcelles de toutes cultures, les bois, les prairies ou les zones urbanisées. En 2015, nous avons cartographié le paysage dans une zone circulaire de 2 000 m autour des parcelles échantillonnées et en 2016 nous avons cartographié toute la zone à partir de relevés d'occupation du sol sur le terrain. La cartographie de 2015 est présentée dans la Figure 8, celle de 2016 dans la Figure 9.

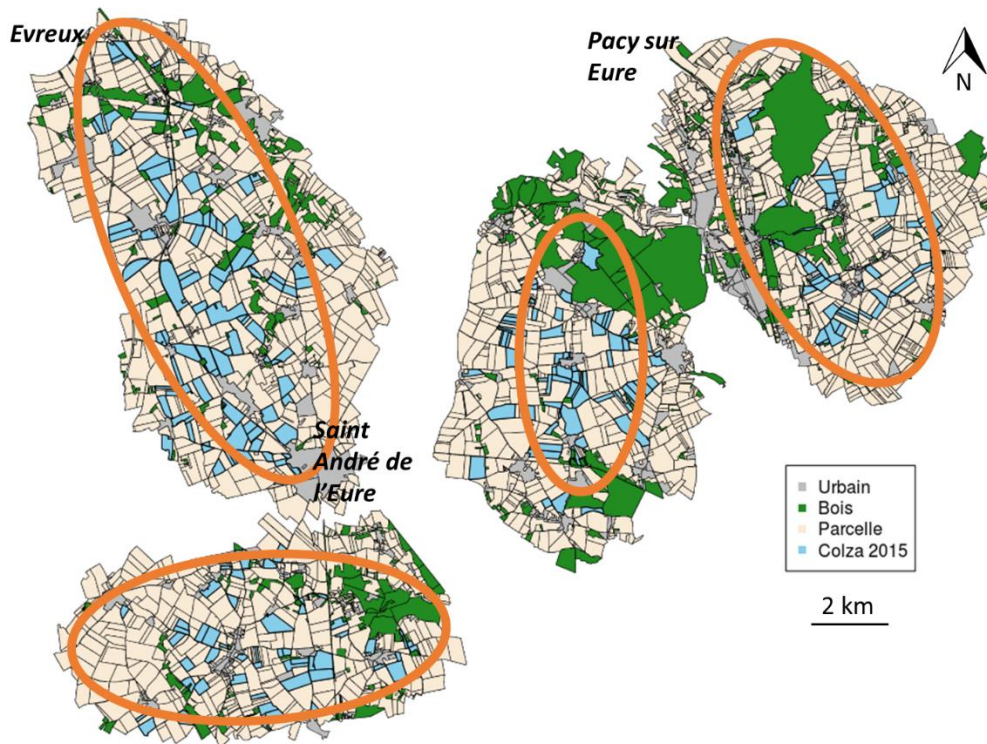


Figure 8 : Cartographie de la zone d'étude en 2015

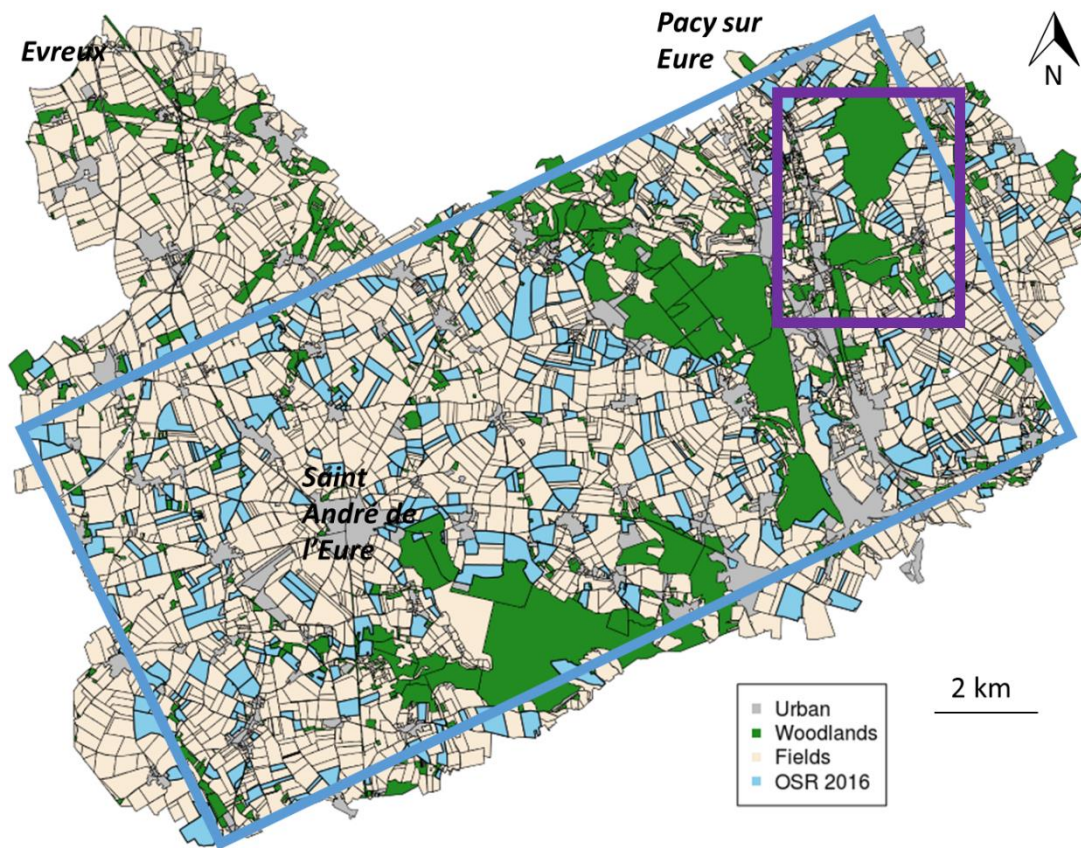




Figure 9: Cartographie de la zone d'étude en 2016

### III. Sélection des parcelles en 2015 et 2016

Nous avons exploité cette diversité de paysage pour

- (i) tester sur une large zone les influences du paysage soit sur la structure génétique des méligèthes (chapitre 1) soit l'abondance des parasitoïdes (chapitre 6) : c'est la zone en orange sillonnée en 2016
- (ii) quantifier plus précisément sur une zone réduite la distance parcourue par les méligèthes lors de la première phase de dispersion (chapitre 3) et le rôle des habitats comme les prairies, bords de champs et de bois pendant leurs deux phases de dispersion (chapitre 4) : il s'agit de la zone violette. Ici, les habitats semi-naturels ont été échantillonnés, des tentes à émergence dans les bois ont été installées, 5 parcelles étaient concernées pour le suivi dans les parcelles de colza. C'est sur cette zone que l'apparement entre méligèthes a été testé (chapitre 2).

En 2015, nous avons sélectionné 4 zones présentant un gradient d'habitats semi-naturels (les 4 zones en orange dans la figure 1). Vingt-quatre parcelles de colza ont été choisies en fonction de la complexité du paysage (i.e. la proportion en habitats semi-naturels dans le paysage), la distance entre les parcelles et de manière à représenter ces 4 zones de manière équivalente (4 à 7 parcelles par zone). Dans deux des zones (zones 2 et 3), les parcelles ont été choisies suivant un transect partant du bois. Ces 24 parcelles ont servi au chapitre 2. En 2016, dans le but de couvrir entièrement la zone d'étude, 100 parcelles de colza ont été sélectionnées. Cette zone est représentée par le carré bleu de la figure 1 et a servi aux chapitres 1, 3, 4, 5, 6.

### IV. Echantillonnages dans la zone d'étude

#### I. Echantillonnage des méligèthes adultes dans les parcelles

Pour comprendre la dispersion des méligèthes au printemps et estimer leur distance moyenne de dispersion, nous avons échantillonné des méligèthes par battage ou beating dans 24 parcelles en 2015. Le beating consiste à secouer les hampes de colza dans des sacs pour faire tomber les méligèthes. Cet échantillonnage a été réalisé à 5 dates entre mars et avril. Ces données ont servi à mesurer la dispersion des méligèthes dans le chapitre 2. Cet échantillonnage a été répété en 2016 dans 5 parcelles de colza et a servi en tant que populations de l'Eure au chapitre 1.

## ***II. Echantillonnage de la nouvelle génération de méligèthes***

Pour suivre une génération de la naissance des individus à leur reproduction, nous avons échantillonné des méligèthes dans les parcelles de colza au moment où ils émergent (tentes à émergence), dans les bois (tentes à émergence), dans les prairies (prélèvement des individus par beating) et de nouveau dans les parcelles de colza (beating). Ces échantillonnages ont aussi servi à d'autres études, c'est pourquoi ils sont détaillés dans les différentes parties ci-dessous.

Pour capturer les méligèthes de la nouvelle génération, émergeant des parcelles de colza, nous avons placé des tentes à émergence dans ces parcelles. Les tentes à émergence sont des tentes asymétriques servant à collecter l'ensemble des insectes émergeant du sol sur une surface d'environ 1.2 m<sup>2</sup> (Figure 10). La totalité des insectes est collecté au point le plus haut de l'arête faitière dans une tête de collecte contenant de l'éthanol à 90 % pour tuer et conserver les individus. Les pièges ont été relevés 4 fois à une semaine d'intervalle entre chaque relevé et les insectes identifiés et dénombrés au laboratoire. Dans 4 des 24 parcelles en juin 2015, 4 de ces tentes à émergence ont été placées pour capturer la nouvelle génération émergente. Ces données ont servi de point de départ pour suivre une génération et trouver des apparentés dans le chapitre 2.



*Figure 10: Tente à émergence dans une parcelle de colza en 2015. Photo : A. Butier*

### ***Echantillonnage des méligèthes dans les bois***

Des tentes à émergences plus grandes ont été placées dans les bois proches des parcelles échantillonnées de février à avril 2016 pour capturer les méligèthes sortant de diapause (Figure 11). Ces tentes ont été relevées toutes deux semaines et toutes les semaines lors des pics d'abondance. Ces données ont servi à suivre la génération du chapitre 3.



Figure 11: Tente à émergence dans un bois en 2016. A. Butier

### **III. Echantillonnage de méligèthes dans les fleurs de tous types d'habitats**

Pour comprendre où vont les méligèthes lors des deux phases de dispersion et sur quelles fleurs ils vont, des échantillonnages ont été réalisés dans des prairies, des bords de parcelles, des friches et dans l'intérieur des parcelles. Ces habitats ont été sélectionnés autour des parcelles de colza échantillonnées en 2016. Dans chacune de ces surfaces, le recouvrement et le nombre de fleurs ont été mesurés et les méligèthes ont été capturés dans 5 quadrats de 60 \* 60 cm. Ces mesures ont été effectuées en mars et avril dans les prairies, les friches et les bords de parcelles et en juin dans ces trois types de surfaces en ajoutant les parcelles d'autres cultures. Ces données ont été utilisées pour le chapitre sur l'apparement (chapitre 2) ainsi que pour le chapitre 4 sur l'étude des relais lors de la dispersion des méligèthes.

### **IV. Echantillonnage de parasitoïdes dans différents habitats**

Nous avons par ailleurs durant l'année 2016 essayé de capturer des parasitoïdes dans les prairies de la zone C. 52 pièges jaunes (gobelets) ont été placés dans des prairies et 18 cuvettes jaunes dans des parcelles de colza avant l'émergence des parasitoïdes, c'est-à-dire fin avril. Ces pièges ont été relevés toutes les semaines jusque fin mai. Des parasitoïdes ont été trouvés dans les cuvettes dans les parcelles de colza mais aucun n'a été trouvé dans les pièges des prairies. De même, nous avons échantillonné à l'aide de filets fauchoirs des parasitoïdes dans les mêmes surfaces début mai et nous n'avons capturé aucun parasitoïdes dans les prairies mais plusieurs dans les parcelles de colza.

### ***V. Echantillonnage de méligèthes couplé aux parasitoïdes***

Pour étudier la structure génétique et les liens entre les abondances de méligèthes et de parasitoïdes, des échantillonnages ont été réalisés dans les 100 parcelles en 2016. Dans chacune des parcelles, nous avons échantillonné 15 fois 5 hampes florales pour capturer des méligèthes, des larves de méligèthes et des parasitoïdes adultes. Cet échantillonnage a été réalisé une fois en mai 2016. Ces données ont servi aux articles des deux chapitres sur les parasitoïdes (chapitre 5 et 6), ainsi que pour le suivi de la génération de méligèthes (chapitre 2).

### **V. Echantillons en France et en Europe**

Pour les analyses de génétique à l'échelle de la France et de l'Europe des chapitres 1 et 5, les méligèthes et les parasitoïdes ont été prélevés par Terre Inovia et par des chercheurs de la communauté européenne. Ici, un échantillon correspond à environ 30 individus échantillonnés dans une seule parcelle par battage.

L'ensemble de tous ces échantillonnages et de leur utilisation dans les différents chapitres sont présentés dans le Tableau 1 : Table 1 Récapitulatif des différents échantillonnages et de leur répartition par chapitre.

## **VI. Extraction d'ADN**

Puisque nous avons différents types d'échantillons, c'est-à-dire des méligèthes et des parasitoïdes à différents stades, nous avons réalisé de nombreux tests d'extraction d'ADN pour qu'ils soient adaptés au type d'échantillon.

### ***I. Méligèthes et parasitoïdes adultes***

L'extraction d'ADN des adultes parasitoïdes et méligèthes a été réalisée dans des plaques à 96 puits. Chaque individu a été placé dans un des puits avec 50 µl d'eau et une bille d'acier de 2 mm et ont été broyés à l'aide d'un 1600 MiniG (Spex® SamplePrep) tissue homogenizer à 1500 coups/min pendant 30 secondes. A chacun de ces échantillons ont été ajoutés 50µl d'une solution contenant du Chelex 100 (Biorad) à 20 % et 6 % de protéinase K (Eurobio). Nous avons extrait l'ADN des insectes de cette manière aux chapitres 1 et 2 puis 5.

### ***II. Œufs isolés de parasitoïdes***

Le même protocole que sur les parasitoïdes adultes a été testé sur les œufs isolés de parasitoïdes mais l'ADN était trop dilué. L'ajout d'eau et l'étape de broyage ont été supprimés et seulement 30 µl du mélange de chelex/protéinase K ont été ajoutés aux œufs. Nous avons extrait l'ADN des œufs de cette manière pour le chapitre 5.

## Matériel et méthodes

Tableau 1 : Table 1 Récapitulatif des différents échantillonnages et de leur répartition par chapitre

Zones	Chapitre 1 Structure des méligèthes	Chapitre 2 Apparement des méligèthes sur tout le cycle	Chapitre 3 Distance de dispersion des méligèthes	Chapitre 4 Fleurs sauvages	Chapitre 5 génétique des parasitoïdes	Chapitre 6 Parasitoïdes et paysage
A: 2015		Sous-échantillon de 4 parcelles sur les 24 621 méligèthes Habitat échantillonné : Beating dans fleurs de colza, au sol dans parcelles de colza par tentes à émergence	24 parcelles 3657 méligèthes Habitat échantillonné : fleurs de colza par beating			
B : 2016	Sous échantillon de 6 parcelles sur les 96 141 méligèthes Habitat échantillonné : fleurs de colza par beating				96 parcelles 128 parasitoïdes, adultes 92 parasitoïdes, œufs habitat : fleurs de colza par beating	96 parcelles 5151 méligèthes, adultes 7921 méligèthes, larves 138 parasitoïdes, adultes 340 parasitoïdes, œufs habitat : fleurs de colza par beating
C : 2016		Bois : 129 méligèthes, piégés dans des tentes Prairies : 172 méligèthes dans fleurs de prairies par beating 5 parcelles de colza : 360 méligèthes dans fleurs de colza par beating		17 parcelles, 14 prairies, 23 bords, 6 friches 4 159 méligèthes habitat : captures directe dans les fleurs prairies, colza, friches, parcelles autres, bords de route		18 cuvettes jaunes dans les parcelles de colza 52 pièges jaunes dans les prairies 0 parasitoïde dans les prairies 78 parasitoïdes dans les colza Habitats : prairies et colza
Europe	12 parcelles de colza réparties dans les autres pays 317 méligèthes				5 parcelles de colza 111 parasitoïdes	

### **III. Larves de méligèthes**

Une étude de génétique du paysage sur les populations de méligèthes de l'Eure récoltées en 2016, sur l'ensemble des 100 parcelles aurait pu apporter des éclaircissements sur l'effet du paysage sur les populations de méligèthes. Plusieurs raisons ont conduit à la non réalisation de cette étude. Nous avons réalisé de nombreux tests infructueux d'extraction d'ADN de ces larves. Ainsi, nous avons tenté différentes concentrations de Chelex sur larves entières, des larves broyées ou seulement leur tête, mais aussi des extractions au CTAB (Vroh Bi et al., 1996). Ces extractions d'ADN infructueuses étaient probablement dues à la conservation de l'ADN des larves qui se fait plus difficilement que pour des adultes. En effet, ces larves ont été extraites de fleurs de colza après avoir été congelées à -20°C. Les changements répétés de température ont pu dégrader l'ADN. Face à ces problèmes nous aurions pu utiliser les adultes capturés en même temps que les larves. Cependant, des problèmes de coût et de temps sont apparus. Nous avons préféré favoriser l'appareillage sur une petite zone spatiale pour augmenter nos probabilités de trouver des apparentés. Ces analyses sur larves de méligèthes capturées dans les 96 parcelles de 2016 n'ont donc pas pu être mobilisées dans ce manuscrit. Seules les méligèthes adultes ont été mobilisées dans les chapitres 1 et 2.

## **VII. Analyses génétiques**

### **I. Analyse de la diversité génétique**

Nous avons analysé la diversité génétique des méligèthes et des parasitoïdes européens. Pour mesurer l'hétérozygotie attendue ( $H_E$ ) et observée ( $H_O$ ) nous avons utilisé le logiciel Genepop version 4.2.2 (Rousset et al 2008). Le test exact de ce logiciel a été utilisé pour mesurer la déviation à l'équilibre d'Hardy Weinberg, ainsi que l'indice de fixation, FST (Weir et Cockerham, 1984) pour les deux espèces. Le déséquilibre de liaison et le taux d'allèles nuls ont aussi été calculés avec ce logiciel.

La richesse allélique, c'est-à-dire le nombre d'allèles réajusté en fonction du nombre d'individus par population a été calculé avec le logiciel HP-Rare.

Enfin, pour détecter si les populations de méligèthes sont en expansion, le logiciel BOTTLENECK a été utilisé (Piry et al., 1999).

### **II. Analyse de la structure génétique**

La structure génétique des populations européennes de méligèthes et de parasitoïdes a été analysée en utilisant le logiciel STRUCTURE version 2.3.4 (Pritchard *et al.*, 2000). Nous avons

estimé le nombre optimal de groupes  $K$  dans lesquels pouvaient être assignés des individus en testant la vraisemblance de modèles ayant des valeurs de  $K$  allant de 1 à 10. Pour chaque modèle, nous avons réalisé 10 runs de 500 000 itérations après une étape de « burn-in » de 200 000 itérations. Ces assignations d'individus ont été calculées en assumant une admixture entre les  $K$  groupes. Comme recommandé par Wang (2016), la localisation géographique de nos échantillons et la contribution non égale de chaque groupe  $K$  à l'admixture ont été utilisés comme prior.

### **III. Assignation de parentés**

Des assignations de parentés ont été réalisées pour les deux espèces avec le logiciel COLONY (Jones & Wang, 2010). Nous avons utilisé la méthode FL-PLS combined (Full-Likelihood/Pair-Likelihood Score). Cette méthode est recommandée pour détecter des paires de frères dans de larges jeux de données. La méthode Full likelihood étant trop chronophage et la méthode Pair-likelihood imprécise (Wang, 2012).

### **VIII. Analyses statistiques et bayésiennes**

Les mesures d'abondances de méligèthes et de parasitoïdes des deux années d'échantillonnage ont été utilisées dans deux types de modèles. Nous avons utilisé des modèles fondamentaux tels que des modèles linéaires généralisés. Dans ces modèles, une loi négative binomiale a été utilisée, nos données étant surdispersées. Ce type de modèle a été utilisé pour mesurer l'effet des différents habitats sur les abondances de méligèthes, ainsi que pour mesurer l'effet du paysage sur les abondances de parasitoïdes adultes.

Ensuite, nous avons considéré l'arrivée des méligèthes dans des endroits explicites, comme par exemple les colzas ou les habitats semi-naturels. En allant plus loin qu'en utilisant des régressions, nous avons écrit des modèles adaptés à ce qu'on connaît de la dispersion des méligèthes et ajusté ces modèles avec des Chaines de Monté Carlo Markov. C'est notamment le cas dans le chapitre 3 où on cherche à estimer la distance de dispersion moyenne des bois vers les colzas. C'est aussi le cas dans la deuxième partie du chapitre 4 où on identifie les fleurs attractives au sein d'un quadrat pour comprendre comment se distribuent les méligèthes en arrivant dans un quadrat sur les différentes espèces de fleurs.

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## Chapitre 1

Développement de marqueurs  
microsatellites et analyse de la structure  
génétique des populations de melligèthes,  
*Brassicogethes aeneus* en Europe, en  
France et dans une région, l'Eure



## **Chapitre 1 : Développement de marqueurs microsatellites et analyse de la structure génétique des populations de méligèthes, *Brassicogethes aeneus* en Europe, en France et dans une région, l'Eure**

L'objectif de ce chapitre est d'appréhender la dynamique des méligèthes à partir de l'analyse de la différenciation génétique entre populations à différentes échelles spatiales.

Pour cela, nous avons développé des marqueurs microsatellites pour les méligèthes et analysé la variabilité génétique de 18 populations : six localisées dans l'Eure, sept en France dans d'autres bassins de production de colza et cinq dans d'autres pays européens : l'Autriche, l'Estonie, la Pologne, la Suède et la Suisse. Chaque population de méligèthes correspondait à un échantillon d'individus collectés dans une même parcelle de colza. Nous avons estimé la diversité génétique dans chaque population, ainsi que leur structuration génétique.

Ce travail fait l'objet du chapitre 1 et correspond à un article soumis en août 2017 à la revue *Pest Management Science* et intitulé : "***Limited genetic structure of Brassicogethes aeneus populations in France and in Europe***".



## Limited genetic structure of *Brassicogethes aeneus* populations in France and in Europe

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Isolation and characterization of microsatellite loci in the pollen beetles, *Brassicogethes aeneus*, and analysis of the genetic diversity and structure of 18 European populations.

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### Abstract

**BACKGROUND:** The pollen beetles, *Brassicogethes aeneus* (Fabricius, 1775), is one of the most significant pests of oilseed rape. To shed light on its dispersal abilities, we developed microsatellite markers and analyze the genetic structure of six populations in Eure, France, seven other in France, and five in other European countries.

**RESULTS:** The genetic variability was similar among the 18 population samples. Seven populations showed a significant deficit in heterozygosity compared with the expected at mutation drift equilibrium, suggesting a demographic expansion, likely corresponding to the development of oilseed rape culture in the last millenary. The genetic differentiation among population samples was significant in Europe (global  $F_{ST} = 0.008$ ) but not in France ( $F_{ST} < 0.003$ ). All individuals were assigned to one genetic cluster (Structure) but isolation by distance was significant, likely reflecting pre-agrarian gene flow.

### CONCLUSION:

The interpretation of genetic analysis results is not straightforward given the size of the populations and the complexity of the demographic history of such a non-invasive crop pest. Nevertheless, as the gene flow, even in the small pre-agrarian populations, was important enough to prevent the creation of genetic clusters, the management of pollen beetles should likely be thought at the European scale. **Keywords:** *Brassicogethes aeneus*, genetic diversity, genetic structure, microsatellites, oilseed rape, pollen beetles

## 1. Introduction

The pollen beetle (*Brassicogethes aeneus* (Fabricius, 1775), formally named *Meligethes aeneus* (Fabricius, 1775) is one of the major pests of oilseed rape (OSR) crop in Europe [1]. After their emergence from overwintering areas, especially woodlands, adults migrate to OSR fields to feed on pollen and oviposit in buds thereby inflicting severe yield losses [2]. The new generation emerges in the summer and seeks overwintering sites. The pollen beetles thus have two important dispersal phases in their life cycle.

The cultivated area of OSR in France has greatly increased since the 1980's, in part due to the value of this crop as a biofuel. OSR crops receive a large amount of pesticides in a majority of countries [3] but resistance to several products such as pyrethroids appeared particularly in pollen beetles [4] [5]. Insecticide resistance is genetically determined and understanding the gene flow and population structures of insect pests could help manage resistance and deploy alternative control strategies at landscape scale [6].

A first study on pollen beetle sampled from 2001 to 2004 using amplified fragment length polymorphism (AFLP) suggested a high level of gene flow between pollen beetle populations in Sweden [7], but strong structuration of European populations with insects sampled in 2004 [8]. Since then, using mitochondrial DNA of samples collected in 2011, specifically a 797 bp fragment in the cytochrome oxidase 1 gene, five phylogenetic groups in Europe were identified: one in England and Wales, another one in South Eastern Europe from Italy, to Romania and Greece, a third one in North Western Europe (France, Germany, Belgium) a fourth in the Baltic and Scandinavian countries and a fifth, in Scotland that did not significantly differ from the fourth [9]. These studies suggest fairly limited exchanges between pollen beetles European populations. Different genetic markers have different sensitivities or shed a different light on the population structure. Using the AFLP allow to screen a large portion of the genome as one can usually use several hundreds of them at the same time. Only ten to thirty relevant microsatellite loci can be used simultaneously but they are codominant, and are more robust and to assess genetic variability and gene flow between populations [10].

In the present study, we developed 12 relevant microsatellite markers and describe the genetic structure of pollen beetle populations from regional to European spatial scales to assess the genetic dynamic of pollen beetle populations, in the context of large and recently growing populations.

## 2. Material and Methods

### 2.1. Study sites and insect sampling

Pollen beetle adults were collected in 18 fields organized at three nested spatial scales: six OSR fields in the Eure department in France (Figure 1.1a), seven fields from various other regions in France (Figure 1.1b) and five fields from five other European countries (Figure 1.1c). Hereafter, each field sample was considered as a different pollen beetle population. Pollen beetle were collected by beating in one point of each field. Collection took place in spring 2015 with the exception of the population sample from Switzerland (spring 2014) and of the six French samples from Eure (spring 2016). All individuals were identified at species level by comparing their meta-femur for females [11]. Most pollen beetles were identified as *B. aeneus* (99%) except the individuals collected at location 12 (70% were *B. aeneus* and 30% were *B. viridescens*). Hereafter, we only used *B. aeneus* individuals unless otherwise specified.

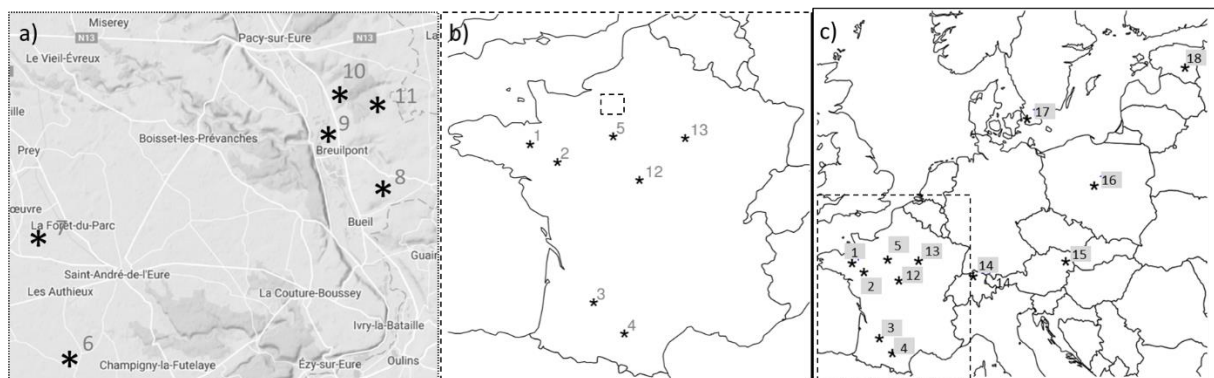


Figure 1.1 Geographical positions of the 18 *B. aeneus* population samples. a) Population samples from the Eure department. b) Population samples from France. c) Population samples from other countries in Europe. Details on the population locations are in Table 2.

### 2.2. DNA extraction

DNA extractions were performed in a 96-well format plate. First, pollen beetle tissues were ground in 50  $\mu$ L of H<sub>2</sub>O using a 2 mm steel bead using a 1600 MiniG (Spex® SamplePrep) homogenizer at 1500 strokes/min for 30 seconds. Second, tissues were digested at 56°C for 14 hours in a 100  $\mu$ l solution including 10% Chelex® 100 (Biorad) and 3% proteinase K using a Mastercycler thermocycler (Eppendorf). The tissue digestion was stopped by a final thermocycler step of 30 min at 98°C. Finally, the supernatant of this solution was used as DNA template for the PCR reactions.



### **2.3. Microsatellite development**

An enriched DNA library with microsatellite sequences was produced following [12] on 9 pollen beetles from three different oil seed rape fields in France. The DNA library was produced by GenoScreen (Lille, France) using Roche 454 GS-FLX Titanium pyrosequencing. A total of 255 microsatellite markers were identified out of the 93,675 DNA sequences obtained (0.3%). The 48 longest microsatellite markers were selected and a primer pair was chosen for each of them using Primer3 [13]. Each forward primer was tailed with a labelled M13 sequence [14]. PCR amplifications were carried out independently for each microsatellite locus in a 12 µl reaction volume containing 1X GoTaq® Flexi Buffer, 1.5 mM MgCl<sub>2</sub>, and 0.1 mg/ml Bovine Serum Albumin, 200 µM of each dNTPs, 0.4 µM of reverse primer, 0.4 µM of labelled M13 forward primer, 0.04 µM of M13-tailed forward primer, 1 unit of GoTaq® Flexi DNA Polymerase (Promega) and 2 µl of DNA template. The PCR conditions were: 5 min at 95°C followed by 30 cycles at 95°C for 30 s, 54°C for 45 s, and 72°C for 45 s, followed by 10 cycles at 94°C for 30 s, 50°C for 45 s, and 72°C for 45 s with a final extension step at 72°C for 10 min.

We tested PCR amplification at these 48 microsatellite loci on six *B. aeneus* individuals. For the loci that amplified, we verified their variability and usefulness for population genetic studies on 24 additional *B. aeneus* individuals from location 1 (Fig. 1). For each microsatellite locus, we calculated the number of alleles, the observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosities. We estimated the frequency of null alleles following Brookfield (1996). We tested deviations to Hardy-Weinberg equilibrium (HWE) using Genepop, version 4.2.2 [15]. We selected the polymorphic loci with less than 10% of null allele and at HWE.

The selected microsatellite loci were combined in PCR multiplex, labelling each forward primer with a fluorescent dye at their 5'-end, either 6-FAM (6-carboxyfluorescein), or HEX (hexachloro-fluoresceine), or TAMRA (carboxy-tetramethyl-rhodamine), or ATTO 565 (Rhodamine dyesclass) (Table S1.1). These loci were also tested on nine *B. viridescens* individuals from location 12 to verify interspecific cross-amplification.

### **2.4. Microsatellite analysis of *B. aeneus* populations**

A total of 433 pollen beetle individuals from 18 populations were genotyped at the selected microsatellite loci (Table 2); note that among the 45 individuals of the location 1, 24 were already genotyped for the microsatellite loci selection. PCR amplifications were carried out with a Mastercycler thermocycler (Eppendorf) in a 10 µl reaction volume containing 5 µl of

master-mix (QIAGEN), 2 µl of primer-mix (primers concentration ranging from 0.13 to 2.5 µM, Table S1), and 2 µl of DNA template. The PCR conditions were: 15 minutes at 95°C followed by 35 cycles at 94°C for 30 s, 54°C for 90 s, and 72°C for 1 min with a final extension step at 72°C for 20 min.

Each PCR multiplex product was diluted in 40 µl H<sub>2</sub>O. Two microliters of this dilution was added to 7.8 µl of HiDi formamide, and 0.2 µl GeneScan™- 600 LIZ® Size standard (Applied Biosystems). This was injected on an ABI 3730xl DNA Analyzer using POP7 polymer (Applied Biosystems). Genotypes were scored using GeneMapper®, version 4.1 (Applied Biosystems).

### 2.5. Analysis of the genetic data

Genetic variability at the selected microsatellite loci was analyzed in 18 pollen beetle field populations across Europe. For each population, we calculated  $H_O$ ,  $H_E$  and estimated the multilocus inbreeding coefficient ( $F_{IS}$ ). We estimated the genotypic differentiation between all population pairs ( $F_{ST}$ ) (16). Exact test for Hardy-Weinberg equilibrium (HWE), for population differentiation from the  $F_{ST}$  and for genotypic disequilibrium among pairs of loci were performed using Genepop 4.2.2 with a significance level of 0.05 for the p-value ( $p$ ). Allelic richness in each population sample was estimated using a rarefaction method [17], using the HP-RARE program [18] parameterized with the smallest population size as the reference. Comparison of allelic richness between populations was tested with a Mann-Whitney-Wilcoxon Test.

We measured the isolation by distance between pairs of population samples as the slope  $b$  of the linearized  $F_{ST}$  transformation  $F_{ST}/(1-F_{ST})$  given the logarithm transformation of the geographical distance [19]. The isolation by distance was tested at different spatial scales using either *i*) all the pairs between the 18 population samples, or *ii*) all the pairs between the 18 population samples except the Estonian sample, or *iii*) the pairs between the 13 French population samples or *iv*) only the pairs between the six Eure population samples (Fig. 1). Indirect genetic estimates of the distance of migration per generation  $\sigma$  and of the population density  $D$  were independently calculated based on  $b$  estimates ( $b=1/4D\pi\sigma^2$ ), assuming the populations were at genetic-drift equilibrium and migration was isotropic in the two spatial dimensions [19]. The range of variation on  $b$  was calculated based on several measure of the isolation by distance including or discarding the Eure and Estonian population samples that were at the more extreme geographical distances (locations 6-11 and 18 respectively; Fig. 1). Estimates on  $\sigma$  were performed using different ecological estimates of *B. aeneus* population

density based on the compilation of observations on the number of pollen beetles per OSR plant (France: [20]; Estonia: [21]; Germany: [22], on density of plants in OSR crops [23] and on density of OSR fields in Europe [24]. Mean, minimal and maximal estimates of current *B. aeneus* population density were calculated multiplying these three different sources of data. Estimations on  $D$  were performed using ecological measures of *B. aeneus* dispersal distances (mark-recapture data) at different period of their cycle between OSR crops and woodland plots [25] and between woodland plots and OSR crop [26]. Mean, minimal and maximal estimates of the parent-offspring dispersal distances were calculated adding the mean, minimal, and maximal values reported in these two papers.

The gene diversity ( $H_E$ ) observed at a locus within a population can differ from the gene diversity expected at mutation-drift equilibrium for the same number of alleles ( $H_{eq}$ ) due to demographic changes [27]. To test if a change of size in *B. aeneus* populations happened in Europe, the observed gene diversities ( $H_E$ ) were compared to the gene diversity values expected at mutation-drift equilibrium ( $H_{eq}$ ) under three different mutation models in each population sample. The infinite allele model (IAM) was initially developed to model mutations occurring with allozyme data. Then, the stepwise mutation model (SMM) was developed to deal with the evolution of repeated DNA sequences. Finally, the two-phase mutation model (TPM), a mix of the two previous models keeping the SMM as preponderant was proposed to model the evolution of microsatellite sequences [28]. Gene diversities at the mutation-drift equilibrium were computed using the Bottleneck software for each mutation model [29].  $H_{eq}$  under the TPM were computed using 90% single-step mutations, 10% multiple-step mutations and a variance among multiple steps of 12. Differences between  $H_E$  and  $H_{eq}$  were tested for each population sample with the set of selected microsatellite loci using the two tailed Wilcoxon's signed-rank test.

To detect clusters among the sampled pollen beetles, we used the Bayesian clustering method implemented in Structure version 2.3.4 [30]. We selected the optimal number of groups  $K$  in which individuals should be assigned by testing the likelihood of models with values of  $K$  from 1 to 10 [30]. For each model, we performed 10 runs of 2,000,000 iterations after a 'burn-in' period of 500,000 iterations. Individual assignments were computed assuming admixture among the  $K$  groups and non-equal contribution of the  $K$  sources to the admixture. As recommended by [31], the geographical locations of the sampled individuals and low degree of admixture (initial Dirichlet parameter ALPHA of 0.75) were used as priors to initialize the simulations.

### 3. Results

#### 3.1. Design and selection of microsatellite markers

The DNA library produced using Roche 454 GS-FLX Titanium pyrosequencing provided 93,675 sequences among which 255 were available to design microsatellite markers. Out of the 48 markers designed, 23 amplified on the six pollen beetle individuals tested and were selected for amplification on a reference population of 24 individuals from location 1 (Tableau 1.1). Among these 23 loci, twelve were finally used to study the genetic of pollen beetle populations. First, we discarded two loci that were monomorphic (*Ma-DMBX2*, *Ma-ERC7C1*). Second, we discarded nine additional loci that were not at HWE and that showed proportions of null alleles over 10 % in the reference population (*Ma-C6CAE*, *Ma-C5QWM*, *Ma-D904J*, *Ma-DCGTK*, *Ma-EJCJJ*, *Ma-EL5EB*, *Ma-C2DIM*, *Ma-DJDOQ*, *Ma-EGYLO*).

At these twelve remaining loci, the number of alleles ranged from three to nine (mean = 6.00), the gene diversity ( $H_E$ ) ranged from 0.17 to 0.85 (mean = 0.63) and proportion of heterozygotes ( $H_O$ ) ranged from 0.18 to 0.87 (mean = 0.60) (Tableau 1.1). The proportions of null allele did not exceed 0.09 (mean = 0.03).

Cross-amplifications of these twelve *B. aeneus* microsatellite loci on *B. viridescens* were successful except for *Ma-CEALQ* and all were polymorphs on this second species (Table S1.2).

*Tableau 1.1 Characterization of the genetic variation at 21 polymorphic microsatellite loci based on 24 Brassicogethes aeneus samples collected in a seed rape field at location 1 (Fig. 1b). Names of the selected loci for population genetic use were underlined. Locus Name/GenBank Accession Number (Acc. No.), forward (F) and reverse (R) primer sequences, repeated motif of the sequenced microsatellite allele, sizes of the sequenced microsatellite allele, number of alleles (A), range of allele sizes, proportion of null alleles, proportion of observed and expected ( $H_O$ ) heterozygotes ( $H_E$ ).*

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Locus	Acc. No.	Primer sequences (5'-3')	Repeat motif	Size (bp)	A	Size range	Null alleles	$H_E$	$H_O$
<i>Ma-CEALQ</i>	MF321854	F: TTTCATTAAGCAACCTGTCTG R: GTGAGAGTAAGTTAAAGGCG	(TC) <sub>6</sub>	222	3	210-220	0.00	0.17	0.18
<i>Ma-D3QFM</i>	MF321855	F: GGAGCACGTAGCAGGAC R: GCTGCTGCGTAATTATAGTG	(AC) <sub>8</sub>	103	6	96-112	0.00	0.44	0.43
<i>Ma-DCH30</i>	MF321862	F: CGCAGATCTAAATTCGTGTG R: GTATAGCGAAACAACAAGTGC	(AC) <sub>7</sub>	117	5	111-125	0.00	0.72	0.87
<i>Ma-DDEYS</i>	MF321863	F: GTACACCGAGAGGCTTTGTC R: GACTGTTCGGCTAGTTTTTATG	(CA) <sub>7</sub>	128	5	122-134	0.00	0.62	0.67
<i>Ma-EL7YR</i>	MF321871	F: CAGGATGATTTTCAGTGGAG R: CGGAAGAGTTGTTTTGTATG	(GGA) <sub>8</sub>	190	4	181-190	0.00	0.58	0.71
<i>Ma-ESPVQ</i>	MF321873	F: GTTAGGATATGAATGTTTCTGC R: CATATGCGACATCGTTGG	(CA) <sub>8</sub>	134	9	130-160	0.00	0.79	0.86
<i>Ma-ESVIZ</i>	MF321874	F: GTCAGTTGTTTGGCTTATTG R: CAGGAACCTCGAACAAAGC	(GAA) <sub>7</sub>	110	7	104-116	0.00	0.53	0.50
<i>Ma-EPL2N</i>	MF321872	F: CCGACTTATCAGGTGTATGG R: CGTTCGACGTTGTGTTACC	(GGA) <sub>11</sub>	171	8	177-198	0.03	0.77	0.70
<i>Ma-DQM5T</i>	MF321866	F: GGGAGAGTGATGTACCTTTG R: GGCAAGATAACTCAGATCC	(TG) <sub>9</sub>	153	5	143-153	0.07	0.65	0.52
<i>Ma-EB7XX</i>	MF321867	F: CTTACTCGCTCGTCCTATATC R: CACCTATCGTGCAGATCAC	(AC) <sub>7</sub>	237	8	217-231	0.08	0.85	0.70
<i>Ma-C4QRG</i>	MF321857	F: AAGAGTATAAGTCGTCGAGC R: AGTGGATGTAGAGAAATAGTGG	(CT) <sub>6</sub>	305	5	301-313	0.08	0.65	0.50
<i>Ma-DM3QY</i>	MF321865	F: CATGTAAGCTATTTTGGGACG R: CTATTTGCTTTGCTTGGATGC	(AC) <sub>12</sub>	195	7	183-197	0.09	0.77	0.61
<i>Ma-C6CAE</i>	MF321859	F: CCTCTACGTCATGGTATGG R: CCAAGATTAGGTCCACTCG	(GT) <sub>7</sub>	126	4	121-129	0.23	0.49	0.17
<i>Ma-EGYLO</i>	MF321868	F: TCAAGTCTGACAACCAAAAG R: GTTTCTGATTCGTTCTTGTC	(GA) <sub>7</sub>	172	2	168-190	0.15	0.41	0.22
<i>Ma-C5QWM</i>	MF321858	F: TATTATGCTCCACCATTAGG R: CCTTCAACTGTAATCAAAGC	(AC) <sub>8</sub>	257	4	250-256	0.22	0.67	0.31
<i>Ma-D904J</i>	MF321860	F: GGAGGGTCAGAAGAGTTTG R: GTGTACGTGTTATAAGGCTGTG	(AAG) <sub>7</sub>	143	6	123-139	0.21	0.71	0.36
<i>Ma-DCGTK</i>	MF321861	F: ATCGTAGCCATCTATTGAGC R: TGGACCTCTTGGTATATTGG	(CTT) <sub>12</sub>	162	9	159-180	0.17	0.62	0.36
<i>Ma-EJCJJ</i>	MF321869	F: CCGACAAGTGCATTACG R: CAATAGACACCATCAATTAGG	(AG) <sub>8</sub>	176	5	154-170	0.21	0.70	0.35
<i>Ma-EL5EB</i>	MF321870	F: CATAGTCTGTAAAGCATGTTG R: CAAGAACTTGACACTTAAATC	(TC) <sub>7</sub>	295	3	295-311	0.28	0.51	0.10
<i>Ma-C2DIM</i>	MF321856	F: CCAAGAAAGGGAACAGG R: CAGTAACCATAGCTCGACAC	(AG) <sub>8</sub>	313	3	282-313	0.18	0.46	0.18
<i>Ma-DJDOQ</i>	MF321864	F: ACGTTTAATTAGTTGGTTGG R: TATTGACGAGCTAATTTTGG	(GA) <sub>13</sub>	119	3	153-163	0.24	0.43	0.11

### 3.2. Genetic variability in pollen beetle populations

In total, 433 individuals from 18 OSR fields in Europe were genotyped at the 12 microsatellite loci. The number of alleles per population sample and per locus ranged from 4.8 to 6.5. The only significant linkage disequilibrium was observed between loci *Ma-EL7YR* and *Ma-DCH30* in one population sample (location 5) deemed insufficient to discard one of them.

Multi-locus tests did not reveal significant departure from HWE in any of the 18 population samples. Inbreeding coefficient values ( $F_{IS}$ ) ranged from -0.05 to 0.12, suggesting limited or null inbreeding, the highest values being observed at loci *Ma-DCH30*, *Ma-CEALQ* and *Ma-EL7YR*.

Observed heterozygosities ( $H_O$ ) ranged from 0.54 to 0.67, with a mean value of 0.60 across all loci and populations. Similarly expected heterozygosities ( $H_E$ ) ranged from 0.57 to 0.64 with a mean value of 0.62 across all loci and populations. The mean allelic richness ( $A_r$ ) per population ranged from 4.44 to 5.88 with a mean value of 5.22 across all loci and populations (Table 1.2). No difference in polymorphism was detected between the 18 population samples using  $H_E$ ,  $H_O$  or  $A_r$  ( $p > 0.05$ ), this suggests similar population sizes or density [32] (Table 1.2).

In all the population samples, the mean gene diversities  $H_E$  were lower than the gene diversities  $H_{eq}$  expected for the same number of alleles at mutation drift equilibrium with TPM and SMM and higher than the  $H_{eq}$  calculated with the IAM mutation models. The deficit of gene diversity  $H_E$  in comparison with  $H_{eq}$  was significant in three out of the 18 population samples with TPM model (Tableau 1.2) and in seven populations with the SMM model. No significant excess of gene diversity  $H_E$  in comparison with  $H_{eq}$  was detected with the IAM model.

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Tableau 1.2: Mean genetic variability ( $\pm$  standard deviation) over 12 microsatellite loci in 18 *Brassicogethes aeneus* population samples. Latitude (Lat) and longitude (Long) of the location of the population sample, number of individuals genotyped per population sample ( $N$ ), allelic richness ( $A_r$ ) computed for  $N=16$ , observed heterozygote proportion ( $H_O$ ), gene diversity ( $H_E$ ) and expected gene diversity ( $H_{eq}$ ) at the mutation-drift equilibrium under the Two-Phase mutations (TPM). Bold character indicate  $H_E$  values significantly lower than  $H_{eq}$  under the TPM and underlined characters indicate  $H_E$  values significantly lower than  $H_{eq}$  under the SMM. France (E): Population samples from the Eure department.

Site	Country	Lat	Long	$N$	$A_r$	$H_O$	$H_E$	$H_{eq}$ (TPM)	$H_{eq}$ (SMM)
1	France	48.112	-1.776	45	5.24 ( $\pm 1.83$ )	0.61 ( $\pm 0.20$ )	<u>0.62</u> ( $\pm 0.20$ )	0.70 ( $\pm 0.11$ )	0.72 ( $\pm 0.07$ )
2	France	47.664	-0.789	25	5.42 ( $\pm 2.03$ )	0.65 ( $\pm 0.19$ )	0.63 ( $\pm 0.17$ )	0.69 ( $\pm 0.13$ )	0.70 ( $\pm 0.08$ )
3	France	43.408	1.646	24	5.44 ( $\pm 1.56$ )	0.59 ( $\pm 0.18$ )	0.64 ( $\pm 0.17$ )	0.72 ( $\pm 0.08$ )	0.73 ( $\pm 0.07$ )
4	France	44.18	0.53	24	5.50 ( $\pm 1.61$ )	0.66 ( $\pm 0.15$ )	<u>0.65</u> ( $\pm 0.16$ )	0.72 ( $\pm 0.08$ )	0.73 ( $\pm 0.07$ )
5	France	48.299	1.245	26	5.01 ( $\pm 1.35$ )	0.60 ( $\pm 0.18$ )	0.61 ( $\pm 0.15$ )	0.68 ( $\pm 0.10$ )	0.69 ( $\pm 0.08$ )
6	France(E)	48.864	1.243	25	5.40 ( $\pm 1.75$ )	0.67 ( $\pm 0.21$ )	<u>0.62</u> ( $\pm 0.18$ )	0.70 ( $\pm 0.09$ )	0.72 ( $\pm 0.07$ )
7	France(E)	48.923	1.214	22	5.06 ( $\pm 1.59$ )	0.60 ( $\pm 0.20$ )	<b>0.61</b> ( $\pm 0.17$ )	0.68 ( $\pm 0.08$ )	0.70 ( $\pm 0.08$ )
8	France(E)	48.946	1.465	24	5.63 ( $\pm 2.35$ )	0.63 ( $\pm 0.18$ )	0.63 ( $\pm 0.16$ )	0.70 ( $\pm 0.17$ )	0.71 ( $\pm 0.08$ )
9	France(E)	48.974	1.421	23	5.43 ( $\pm 1.83$ )	0.58 ( $\pm 0.22$ )	0.57 ( $\pm 0.16$ )	0.70 ( $\pm 0.10$ )	0.71 ( $\pm 0.07$ )
10	France(E)	48.987	1.428	23	5.07 ( $\pm 1.74$ )	0.58 ( $\pm 0.19$ )	0.62 ( $\pm 0.16$ )	0.68 ( $\pm 0.12$ )	0.69 ( $\pm 0.08$ )
11	France(E)	48.972	1.448	24	5.23 ( $\pm 1.78$ )	0.54 ( $\pm 0.17$ )	<u>0.59</u> ( $\pm 0.14$ )	0.68 ( $\pm 0.14$ )	0.69 ( $\pm 0.08$ )
12	France	47.217	2.199	16	5.50 ( $\pm 2.43$ )	0.63 ( $\pm 0.23$ )	0.61 ( $\pm 0.22$ )	0.68 ( $\pm 0.17$ )	0.69 ( $\pm 0.09$ )
13	France	48.259	3.845	30	5.08 ( $\pm 1.98$ )	0.55 ( $\pm 0.21$ )	<b>0.58</b> ( $\pm 0.21$ )	0.67 ( $\pm 0.13$ )	0.69 ( $\pm 0.10$ )
14	Switzerland	47.431	8.519	28	5.97 ( $\pm 2.19$ )	0.64 ( $\pm 0.20$ )	<b>0.65</b> ( $\pm 0.20$ )	0.71 ( $\pm 0.16$ )	0.69 ( $\pm 0.11$ )
15	Austria	48.208	16.374	30	5.65 ( $\pm 2.43$ )	0.59 ( $\pm 0.22$ )	0.62 ( $\pm 0.21$ )	0.69 ( $\pm 0.18$ )	0.69 ( $\pm 0.12$ )
16	Poland	52.187	18.809	24	5.22 ( $\pm 1.93$ )	0.60 ( $\pm 0.18$ )	0.62 ( $\pm 0.17$ )	0.68 ( $\pm 0.13$ )	0.69 ( $\pm 0.13$ )
17	Sweden	55.658	13.083	21	5.18 ( $\pm 1.53$ )	0.57 ( $\pm 0.17$ )	0.61 ( $\pm 0.17$ )	0.68 ( $\pm 0.15$ )	0.69 ( $\pm 0.14$ )
18	Estonia	58.343	26.527	24	4.43 ( $\pm 1.54$ )	0.59 ( $\pm 0.22$ )	0.61 ( $\pm 0.22$ )	0.63 ( $\pm 0.13$ )	0.69 ( $\pm 0.15$ )

### 3.3. Genetic differentiation between populations

The global fixation index ( $F_{ST}$ ) was 0.005 over the 18 *B. aeneus* population samples, suggesting weak but significant genetic differentiation among the European populations ( $p < 0.001$ ). Out of the 153 pairwise  $F_{ST}$  values calculated between population samples, only a fifth, 31, were different from zero ( $p < 0.05$ ); they always included the Estonian, Polish or Swedish population samples (Supplementary material; Table S1.3). All the pairwise  $F_{ST}$  values including the Estonian population sample significantly differed from zero ( $p > 0.001$ ), suggesting pollen beetle from Estonia might be an outlier population in comparison with the other genotyped populations. Global  $F_{ST}$  over the Eure population samples and over the French populations samples did not significantly differ from 0 (respectively,  $F_{ST} = 0.001$ ,  $p = 0.79$  and  $F_{ST} = 0.003$ ,  $p = 0.18$ ). Global  $F_{ST}$  over the European countries (grouping all individuals from France in one population) was 0.0082 ( $p < 0.001$ ) or 0.0041 without the Estonian population sample ( $p = 0.0014$ ).

Mantel's test of the isolation by distance revealed significant positive correlations between the genetic differentiation and the geographical distance among all population samples (Figure 1.2) ( $F_{ST}/(1 - F_{ST}) = -0.0125 + \ln(\text{km}) 0.0029$ ,  $p = 0.001$ ). Isolation by distance was significant even when excluding the Estonian population sample ( $F_{ST}/(1 - F_{ST}) = -0.0058 + \ln(\text{km}) 0.0014$ ,  $p = 0.01$ ). Mantel's test among the French and among the Eure population samples were not significant ( $p = 0.20$  and  $p = 0.23$ ). When we discarded the distances between Eure populations from the two first Mantel's tests, we had a slope of 0.006 with Estonian population and of 0.0025 without Estonian population.

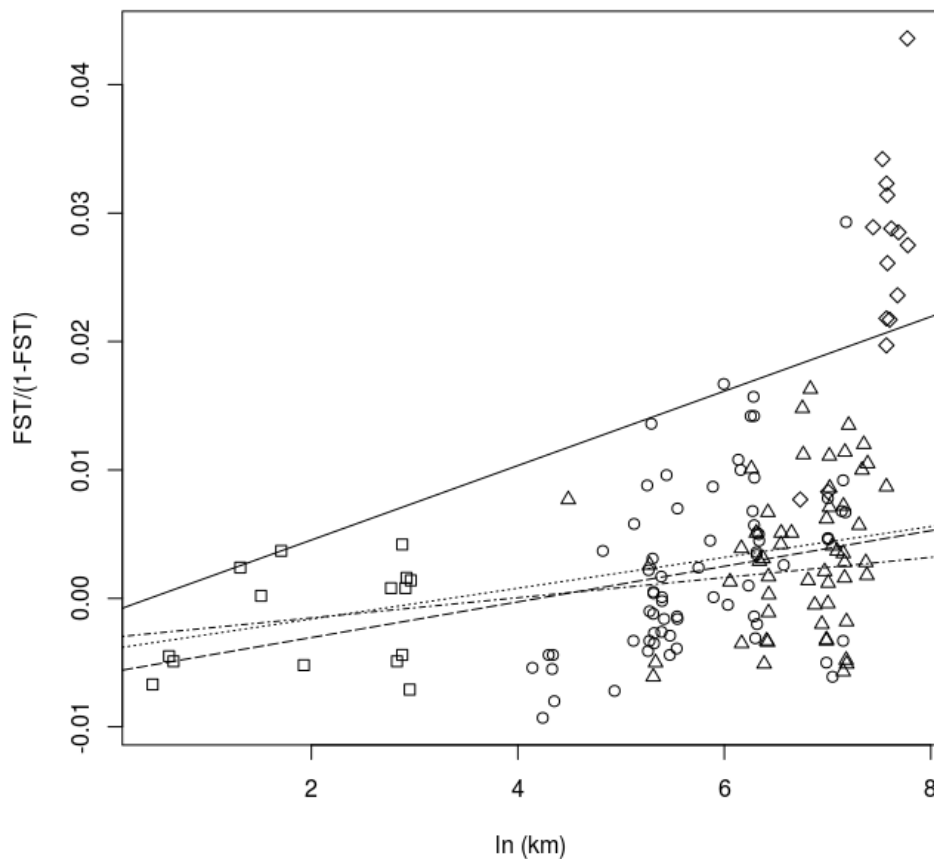


Figure 1.2 Isolation by distance among European populations of *Brassicogethes aeneus*. Squares: pairwise comparison between the Eure population samples, Circles: between the French population samples, Triangles: between population samples from different countries, diamonds: between populations including the Estonian population. Solid line: all populations,  $F_{ST}/(1 - F_{ST}) = -0.0125 + \ln(\text{km}) 0.0029$  ( $p < 0.001$ ). Long dash line: all populations except the Estonian sample,  $F_{ST}/(1 - F_{ST}) = -0.0058 + \ln(\text{km}) 0.0014$ , ( $p < 0.01$ ). Dashed line: French populations, ( $p > 0.05$ ). Dotted line: Eure populations ( $p > 0.05$ ).

Current population density of pollen beetles was estimated at about 2 million individuals per square kilometer on average (range: 150,000 - 4,000,000). Following [19], we calculated the mean distance of migration per generation ( $\sigma$ ) based on the slope estimate of the isolation by distance and the above estimate of density of pollen beetle populations (Tableau 1.3). Using the



range of variation of the population densities estimated from the literature, we estimated the average distance of migration per generation to be  $4.84 \times 10^{-5}$  km possibly ranging from  $2.95 \times 10^{-6}$  to  $2.04 \times 10^{-4}$ .

Dispersal distances per generation were estimated from literature data about 3 kilometers in average (range: 2 – 13). Based on these values, following [19], we calculated an average effective density ( $D$ ) of 3.96 individuals per km<sup>2</sup> with a range of 0.08 to 14.21 individuals per km<sup>2</sup>.

Tableau 1.3 Genetic estimates of the distance of migration per generation ( $\sigma$ ) in km and the effective density ( $D$ ) in number of individuals per km<sup>2</sup> based on four different slope measures of isolation by distance tests and three ecological estimates of  $\sigma$  and  $D$  based on the literature. The first part of the table estimated the  $\sigma$  with ecological estimates of pollen beetle population densities. The second part of the table estimated  $D$  with ecological estimates of dispersal distances.

Ecological Estimates	Estimates according to IBD			
	Without intra Eure distance		With intra Eure distance	
	With Estonia	Without Estonia	With Estonia	Without Estonia
	0.0060	0.0025	0.0029	0.0014
Population density ( $D$ )	Dispersal distance ( $\sigma$ )			
156157 (Estonia)	8.49E-05	2.04E-04	1.76E-04	1.26E-05
1721234 (France)	7.71E-06	1.85E-05	1.59E-05	3.30E-05
4496315 (Germany)	2.95E-06	7.08E-06	6.10E-06	1.26E-05
Dispersal distance ( $\sigma$ )	Population density ( $D$ )			
2	3.32	7.96	6.86	14.21
3	1.47	3.54	3.05	6.32
13	0.08	0.19	0.16	0.34

Based on their microsatellite genotypes, the number of clusters,  $K$ , to which the 433 individuals could be assigned was estimated assuming a uniform prior  $K$  between 1 and 10, using the software Structure. The model that best described the genetic data was for  $K = 1$  ( $p = 1$ ).

#### 4. Discussion

Microsatellite loci are polymorphic and codominant genetic markers and are still widely used for population genetic studies of non-model species [10]. Here, we have designed twelve microsatellite markers for *B. aeneus*, a coleopteran pest for which no such marker were available. These markers were selected among 48 other microsatellite markers from an enriched DNA library using a high-throughput 454 pyrosequencing approach. Relatively few microsatellite markers were useful for genetic study of *B. aeneus* populations in comparison with other coleopteran species: a quarter for the ground beetle *Carabus nemoralis* [33]; a half for the ground beetle *Poecillus cupreus* [34] or the invasive western corn rootworm *Diabrotica virgifera virgifera* [35]. This may reflect high proportions of null alleles at the microsatellite

markers designed in *B. aeneus*. Furthermore, *D. virgifera* and the ground beetle *Poecillus cupreus* have a much larger number of alleles per microsatellite locus than *B. aeneus* (up to 15; [35], [34]). This illustrates that the number of microsatellite sequences initially available does not necessarily correlate with the number of markers useful for population genetics studies.

Eleven of the twelve selected microsatellite loci in *B. aeneus* also amplified (some of them with other alleles) in *B. viridescens* and were polymorphic, potentially allowing population genetics analysis in *B. viridescens* and even genetic differentiation of the two species. Such cross-priming between species is not common. For the microsatellites loci developed on *D. virgifera* *virgifera*, all amplified in *D. virgifera* *zeae*, but only one third amplified in *D. barberi* and one eighth on *D. undecimpunctata* *howardi* [35]. At most loci, some alleles were found both in *B. viridescens* and *B. aeneus* (PCR products overlapped) but at *Ma-EL7YR*, allele sizes were different enough to differentiate the two species. Though additional evaluation of the polymorphism and neutrality of these loci is necessary for *B. viridescens*, they are good candidates for population genetic studies on this species, a less harmful OSR pest in Europe (36) but a serious issue in Canada and in the USA [37].

The genetic diversity was found similar across the different European populations suggesting similar effective population sizes across Europe [32]. The heterozygosity found is comparable to the one found for other agrarian beetle [34].

The 433 genotyped pollen beetles were assigned in a unique cluster according to the Structure analysis, consistent with a representation of a single, large, European population [38], [39]. In agreement with this result, the genetic differentiation revealed by the 12 microsatellites markers was low and not significant among the samples from the French department Eure ( $F_{ST} = 0.001$ ) and among French samples ( $F_{ST} = 0.003$ ). This result is in agreement with the lack of population genetic structure observed in the United Kingdom with mitochondrial DNA [9] and in Sweden with AFLP [7].

At the continental scale, the genetic differentiation over the six European countries was slightly higher ( $F_{ST} = 0.008$ ) than over France ( $F_{ST} = 0.003$ ) but significant. Moreover, isolation by distance was detected only at the European scale, consistently with the lack of detection of clusters by Structure [38] [39].

[9] found an  $F_{ST}$  of 0.1 among European populations using DNA mitochondrial sequences, a value ten times stronger than our estimate with microsatellite loci ( $F_{ST} = 0.01$ ). [8] found an even stronger genetic structure between European populations using AFLP markers (significant  $F_{ST}$  of 0.70). These differences of  $F_{ST}$  estimates across studies are likely to correspond to

differences between the markers used, notably the dominance of AFLP markers and absence of recombination with mitochondrial markers [40]. Differences of  $F_{ST}$  values could also be explained by differences of mutation rate between the genetic markers and an increase of pollen beetle population sizes because of the increase in OSR production in Europe over the last century. Indeed, it must be noticed that the  $F_{ST}$  does not immediately reflect changes in the current gene flow or population sizes, particularly with large population sizes [41].

Such variations of the population size are very likely as pollen beetles need oilseed rape buds for their oviposition [1]. First planted during the middle age in Flanders, OSR became the most important source of vegetal oil until the middle of the nineteenth century in Europe [42]. In France the OSR cultivated area picked at 200 kha in 1862; afterward, OSR oil was increasingly replaced by vegetal oil from colonies and cultivated areas went down almost 20 folds to 11 kha in 1939 [42]. It then went suddenly up with the Second World War: 152 kha in 1950, down again in the 60's: 91 kha in 1960 before going up after independence wars: 391 kha in 1970; those three last estimates of cultivated surfaces are for all oleaginous crop, mostly OSR [42] [43]. The growth then slowed for 10 years until the advent of the erucic acid-free varieties. The surfaces cultivated with OSR went then up 3 and half fold from 460 kha in 1982 to 1600 kha in 2012. One can expect that other northern European countries had a similar evolution of their OSR cultivated surfaces. In Finland the recent raise might have been even more sudden from 10 000 ha in 1973 to 70 000 ha in 1993 [44]. The similar genetic diversity found across European populations suggests similar effective population sizes in Europe [32] and let think that the different European populations we consider had similar demographic histories.

In summary, the OSR cultivated surfaces are in the 2010's at an all-time high. Its apparition is recent on the evolutionary time scale (1000 year or generations of pollen beetle), and the cultivated surfaces went up in France 4, 145, or 8 folds in respectively 40, 80 or 150 years. It is reasonable to think that the population of *B. aeneus* increased not only with the late increase of OSR surfaces but even more drastically since the middle age with the creation of OSR.

The increased reproductive capacity of this species with the availability of OSR might even have reinforced the increase of the population size [44]. Before apparition of widespread resistances in pollen beetle during the 2000's [4], [5], the pesticides might have limited the population size and genetic diversity, as well as increased genetic structuration [45]. Nevertheless, [7] did not found any effect of pesticides on the population structure of *B. aeneus* which can be understood if the reduction in population due to the insecticides was no greater than the potential increase with the availability of the OSR resource.

The successive demographic changes in populations of pollen beetles (expansions and decrease of OSR surface area) could have only marginally affected the genetic diversity of the populations given the temporal shift of the genetic signal, which grows with the population size [27]. For the Bottleneck analysis, results of simulations by [27] with a 100 fold population size increase showed that the maximum deficit in heterozygotes is attained after  $0.025 \times 2 N_e$  generations, where  $N_e$  is the size of the population after the change. Even if we use our minimum estimate for the population density the maximum deficit in heterozygotes should be attained after 7 800 generations much more than even the approximately 1 000 generations since the middle age. Detecting a signal, even weak, so early after the beginning of the mass culture of OSR, is compatible with a major population increase.

The population density estimated with dispersal distances per generation from literature were extremely low comparing to values of population density measured within fields in the literature [20), [21], [22]. This is also reflected by the very small distance of migration calculated using the isolation by distance. Studies using capture marking recapture showed that *B. aeneus* can travel up to ten kilometers in two days in spring [26] and up to three kilometers in the summer [25]. Fitting dispersal kernels to abundance data, the average dispersal distance from overwintering sites to the crops has been estimated at 1.2 km [46]. To understand the apparent discrepancy with direct observation data, it must be pointed out that the genetic estimates are based on the  $F_{ST}$ , and that this index varies with population sizes and migrations [47]. The  $F_{ST}$  could take millions of generations, here years, to reach its equilibrium [47]. As a consequence and like for the Bottleneck analysis, the genetic signal of population structure corresponds to populations before the apparition of OSR. In this case, as populations might have been way less than a 100 times smaller, the observed structure of the population might correspond to relatively important dispersal abilities, as suggested by the high homogeneity of the genetic diversity over Europe and by the direct estimations of adult pollen beetle dispersal previously cited. At any rates, the structure currently observed is expected to decrease if the OSR remains a major cultivated crop.  $F_{ST}$  based signature in pests of crops that have been cultivated for a long time such as wheat [48] should also be weaker than in pests of recently adopted crops. Such weak spatial genetic structure at the continental scale has been reported for the grain aphid *Sitobion avenae* [49] or for the large pine weevil, *Hylobius abietis* [50], two insect species also displaying high dispersal capacities and very large population sizes.

This underline the limitations inherent to estimating the dispersal of insects with genetics methods particularly for crop pests that have very large population sizes and very variable resources. On one hand, the huge population sizes limit the possibility of structuration of the

population and any structuration of the population might be interpreted as a sign of very limited dispersal. On the other hand, the genetic signature changes slowly and all the more with so large population sizes so that fossil genetic structures corresponding to pre-agrarian era might often be preserved, the initial population sizes being completely uncertain. In addition using such genetic metrics to infer the dispersal supposes that it has not been affected by the agrarian multiplication of the resource or by foundation effects linked with the expansion of the crop from its original production basin. As populations increase, the number of migrant between them is likely to increase proportionally. Even the number of generations theoretically necessary to reach an equilibrium is uncertain. The time to attain an  $F_{ST}$  equilibrium is given by [47] only for populations with increasing population structure and decreasing population sizes, the opposite of what is needed for pests. Similarly the simulations presented by [27] are much less detailed for increasing population sizes (only SMM model) and a population size might increase by much more than a hundred with the agrarian transition.

More generally, we found many theoretical and applied genetic studies rightfully concerned with small endangered populations but few concerned with increasing and large populations such as non-invasive crop pests. More work might be needed to characterize the genetic patterns of such populations [51]. There are also limitations more specific to this work. Ideally the population samples should have been collected the same year, nevertheless, the 3 years time span of our study seems negligible compared to the evolutionary times discussed above for the evolution of the genetic patterns in our case. Our spatial sampling was also limited out of France, in particular, Great Brittany and Deutschland are major OSR producers of North-Western Europe.

### **5. Conclusion**

The high variability observed in the 12 developed microsatellite loci indicates they are useful for measuring genetic patterns in populations of *B. aeneus*. We confirmed the previously observed weak population structure of pollen beetles in Europe and found no genetic structure in France. We also found the signature of a recent population increase likely corresponding to the apparition of OSR as a major crop in the middle age. Given the generation times of the pollen beetle and its current population sizes, the genetic structure we observe today likely corresponds to the old structure of wild pollen beetles and should decrease with time. In any cases, given the unity of the population at the European size and the important dispersal according to ecological studies, some gene flow likely occurs at the continental scale, implying that the management of insecticide resistance alleles, such as pesticides interdictions or

diversification and alternative pest management, should be considered at a very large scale. The example of *B. aeneus* also shows how more generally crop pests might represent a major challenge for classical genetic structure analysis given their huge population sizes and their massive and recent variations.

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## Chapitre 1 : Structure génétique des populations de méligèthes à l'échelle continentale

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## Supporting Information

Tableau S.1.1 : Polymorphic microsatellite loci selected in this study with reference to the labelled dye of the forward primer and the molar concentration (C) of each locus each multiplex. 6-FAM (6-carboxyfluorescein), HEX (hexachloro-fluoresceine), TAMRA (carboxy-tetramethyl-rhodamine), ATTO 565 (Rhodamine dyesclass).

Locus ID	Dye	C (nM)
Multiplex 1		
<i>Ma-D3QFM</i>	6-FAM	0.07 $\mu$ M
<i>Ma-ESPVQ</i>	Tamra	0.50 $\mu$ M
<i>Ma-DM3QY</i>	Hex	0.20 $\mu$ M
<i>Ma-C4QRG</i>	Hex	0.15 $\mu$ M
<i>Ma-ESVIZ</i>	Atto-565	0.15 $\mu$ M
<i>Ma-EPL2N</i>	Atto-565	0.30 $\mu$ M
Multiplex 2		
<i>Ma-DDEYS</i>	6-FAM	0.05 $\mu$ M
<i>Ma-DCH30</i>	Tamra	0.25 $\mu$ M
<i>Ma-EL7YR</i>	Tamra	0.50 $\mu$ M
<i>Ma-DQM5T</i>	Hex	0.15 $\mu$ M
<i>Ma-EB7XX</i>	Hex	0.10 $\mu$ M
<i>Ma-CEALQ</i>	Atto-565	0.15 $\mu$ M

Tableau S1.2: Characterization of 12 polymorphic microsatellite loci in 8 *Brassicogethes viridescens* samples collected in a seed rape field at field 14. Sizes of the PCR products, number of alleles.

Locus	Alleles	Size range
<i>Ma-C4QRG</i>	3	302-312
<i>Ma-CEALQ</i>	/	/
<i>Ma-EL7YR</i>	2	175-194
<i>Ma-EB7XX</i>	4	205-238
<i>Ma-D3QFM</i>	3	191-121
<i>Ma-DCH30</i>	3	104-118
<i>Ma-DDEYS</i>	4	110-130
<i>Ma-DM3QY</i>	4	188-198
<i>Ma-DQM5T</i>	5	147-161
<i>Ma-EPL2N</i>	6	158-175
<i>Ma-ESPVQ</i>	5	124-144
<i>Ma-ESVIZ</i>	4	110-125

## Chapitre 1 : Structure génétique des populations de méligèthes à l'échelle continentale

Table S1.3. Pairwise  $F_{ST}$  estimates (left part) and significant  $F_{ST}$  values (right part) between 18 population samples of *Brassicogethes aeneus* (for details on locations and code see Table 1). Bold character indicate significant  $F_{ST}$  values.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1	/	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.05	0.001	NS	0.001	
2	0.0077	/	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.001
3	0.0030	0.0010	/	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.05	NS	0.001	
4	0.0099	0.0164	0.0037	/	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.001	0.05	0.001	
5	-0.0016	-0.0033	-0.0031	0.0107	/	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.05	NS	NS	0.001	
6	-0.0044	0.0031	-0.0033	0.0100	-0.0054	/	NS	NS	NS	NS	NS	NS	NS	NS	0.05	NS	0.05	0.001	
7	-0.0029	-0.0035	-0.0035	0.0067	-0.0094	-0.0053	/	NS	NS	NS	NS	NS	NS	NS	NS	0.001	NS	0.001	
8	0.0070	-0.0026	0.0066	0.0154	-0.0044	0.0042	0.0008	/	NS	NS	NS	NS	NS	NS	NS	0.001	NS	0.001	
9	-0.0039	0.0017	0.0017	0.0140	-0.0055	0.0016	0.0008	0.0002	/	NS	NS	NS	NS	NS	NS	0.05	NS	0.001	
10	-0.0014	0.0001	0.0003	0.0093	-0.0081	-0.0071	-0.0049	0.0037	-0.0067	/	NS	NS	NS	NS	NS	NS	NS	0.001	
11	-0.0016	-0.0002	-0.0011	0.0057	-0.0044	0.0014	-0.0044	0.0023	-0.0045	-0.0049	/	NS	NS	NS	NS	NS	NS	0.001	
12	0.0024	0.0095	0.0013	0.0001	-0.0073	0.0026	-0.0013	0.0134	0.0005	-0.0027	-0.0061	/	NS	NS	NS	NS	NS	0.001	
13	-0.0005	0.0045	0.0044	0.0140	-0.0042	0.0004	-0.0050	0.0088	-0.0033	-0.0010	0.0022	0.0058	/	NS	NS	0.05	NS	0.001	
14	0.0051	0.0051	0.0042	0.0026	0.0036	0.0050	0.0029	0.0051	0.0051	-0.0020	0.0034	-0.0036	0.0087	/	NS	0.001	NS	0.001	
15	0.0134	0.0072	0.0067	0.0113	0.0085	0.0070	0.0110	0.0077	0.0082	0.0012	0.0046	0.0021	0.0161	-0.0051	/	NS	0.05	0.001	
16	0.0099	0.0057	0.0018	0.0104	-0.0052	0.0016	0.0028	0.0091	0.0035	-0.0057	-0.0033	-0.0049	0.0041	0.0014	0.0039	/	NS	0.001	
17	-0.0018	0.0066	0.0028	0.0118	-0.0061	0.0046	-0.0004	0.0062	-0.0032	-0.0034	-0.0051	0.0037	-0.0020	-0.0005	0.0146	-0.0014	/	0.001	
18	0.0277	0.0230	0.0267	0.0418	0.0212	0.0254	0.0305	0.0313	0.0214	0.0194	0.0087	0.0280	0.0331	0.0281	0.0285	0.0077	0.0110	/	



## Chapitre 2

Etude de la structuration génétique  
à l'échelle locale et des distances  
caractéristiques au sein de fratries  
de méligèthes



## **Chapitre 2 : Etude de la structuration génétique à l'échelle locale et des distances caractéristiques au sein de fratries de méligèthes**

Le chapitre précédent a montré que les populations de méligèthes étaient peu structurées à l'échelle de l'Europe et ne fait pas apparaître de structures à l'échelle de la France ou de l'Eure. Pour affiner la description de la structure génétique, nous nous sommes intéressés aux distances génétiques à l'échelle individuelle sur un grand nombre d'individus de la région de l'Eure. Nous avons utilisé des méthodes d'assignation de parentés pour quantifier les distances couvertes par une génération d'apparentés. Nous avons échantillonné des méligèthes de la même cohorte à des moments différents de leur cycle, depuis leur lieu de naissance (dans les champs de colza en juin 2015) jusqu'à leur reproduction (dans les champs de colza en avril 2016). Nous avons également échantillonnés les méligèthes dans leurs lieux d'hivernage (bois en février-mars 2016) puis dans les lieux d'alimentation potentiels (prairies en avril 2016) avant leur reproduction dans les champs de colza. Plusieurs sites ont été échantillonnés à chacune de ces périodes sur un territoire de 40 km<sup>2</sup> environs dans la vallée de l'Eure et les échantillons récoltés ont été génotypés à 13 locus microsatellites. Nous avons estimé dans chacun des sites à chaque période 1) la proportion de paires d'individus qui étaient pleins frères et demi frères à partir des données génétiques recueillies, et 2) la taille efficace des populations correspondant à chacune des situations de collecte.

Ce chapitre est rédigé sous la forme d'un article, cependant des analyses complémentaires pourront être réalisées avant sa soumission à une revue à comité de lecture. Il s'intitulera :  
**« Sibship assignments and effective population size measure of an oilseed rape pest.**

»





## **Sibship assignments and effective population size measure of an oilseed rape pest**

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### **Abstract**

Pollen beetles, *Brassicogethes aeneus* (formerly named *Meligethes aeneus*) is one of the most significant pest of the oilseed rape crop in Europe. We used sibship assignment methods to better understand the local dynamics of pollen beetles in the Eure department, France. To describe the dilution of the proportion of sibship found over a given surface over a generation, we sampled pollen beetles of the same generation at different time from their birth in OSR fields in 2015 to their breeding in OSR fields in 2016. We also sampled them at their overwintering places (woodlands) and potential feeding places (grassland) in which they might stop before reaching their reproduction places. On each date, we sampled pollen beetles at different locations spread in a same area of 6 x 8 km. Based on sibships assignments, we estimated 1) the proportion of full sibs and half sibs at each of the locations, 2) the proportion of full sibs and half sibs captured over the whole generation across sites, 3) the effective population size in each site. Here we show that the effective population size  $N_e$  decrease with time within a generation. These variables were similar among locations in the same dates. We found dyad of full sibs at more than 2 km between locations suggesting that both dispersal phase were over long distances. These results confirm the dispersal capacities of pollen beetles over several kilometers.

### **1. Introduction**

Pesticide use is a major threat for biodiversity and human health (Geiger et al., 2010) and a wide variety of alternatives strategies have been sought, in particular to deal with insect pests of field crops. These strategies go from the use of repellent (Mauchline et al., 2017), to the use of trap crops (Cook et al., 2004) or resistant cultivars (Herve et al., 2014). The implementation of these strategies requires a good knowledge of the biology of the pests and in particular their

dispersal. A way to approach it is to use sibship methods based on individual pairwise assignment (Thompson, 1976).

The pollen beetles, *Brassicogethes aeneus* (Fabricius, 1775) (formerly *Meligethes aeneus*) (Coleoptera: Nitidulidae) is one of the most significant pest of oilseed rape (OSR). As the OSR receive a large amount of pesticides and as pollen beetles are resistant to pyrethroids (Hansen, 2003), other means of control against this species have to be found. To elaborate these alternatives strategies, a good knowledge of the biology of *B. aeneus* is needed for example on their dispersal. Dispersal is indeed key for this species as it lives in different landscape elements at different points of its life cycle. First, the adults lay their eggs in the buds of the OSR in the spring. Then, the larvae develop in the buds and drop to the ground to pupate when the OSR flowers fade. A few weeks later, the adults of this new generation emerge and seek overwintering sites such as woodlands. They emerge in the spring when temperatures reach 12°C and seek breeding sites (i.e new OSR fields), possibly stopping before at feeding sites such as the edges of grasslands or fields (Juhel et al., 2017a, Taimr et al., 1967).

This species is supposed to have high dispersal capacities, it can travel up to ten kilometers in two days in the spring (Taimr et al., 1967) with a mean dispersal distance of 1.2 km (Juhel et al., 2017b) and up to three kilometers in the summer (Stechmann and Schütte, 1976). These distances were confirmed by a low genetic differentiation in Europe (Juhel et al., 2017c, Kazachkova et al., 2008) but also within countries (Juhel et al., 2017c, Kazachkova et al., 2007).

Here, we want to confirm and precise these estimates of dispersal by identifying distances at which siblings might be found and by measuring the effective population size in pollen beetle populations. We performed a genetic analysis over one pollen beetle generation collecting adults between their emergences in OSR field to their breeding site in OSR field the next year. We also sampled them in several locations at each of the four different dates. Based on sibship assignment method, we estimated the number of full sibs and half sibs and the effective population size  $N_e$  in each of these locations.

## 2. Materiel and Methods

### 2.1. Study site and sample collections

Pollen beetle adults were sampled in the valley of the Eure River (Figure 2.1, Tableau 2.1). Pollen beetle were collected on different dates and at different locations on each date. First, pollen beetles were caught when they emerged from four OSR fields in June 2015 using

## Chapitre 2 : Structuration génétique des méligèthes à une échelle locale

emergence traps (four emergence tents per field). Then, overwintered individuals were caught in woodlands from February to April 2016 using emergence traps (53 emergence traps in different locations along woodland edges). Finally, in April 2016, pollen beetles were caught by beating plants in seven grassland plots (five 60 cm x 60 cm quadrats in each plot either in grasslands or along road borders) and in five OSR fields (10 OSR plants from 10 different points in each of five different fields). All the sampled insects were stored in 90° ethanol solution until DNA extraction. We selected all the individuals sampled in the grasslands, roadsides and woodlands. We kept 50 individuals from each of the OSR tents (or less if we had less than 50 individuals) and 10 individuals from each of the beating in the OSR samples. Distances between pairs of pollen beetle were calculated based on the geographical coordinates at the various sampling points. We used the coordinates transformed in UTM to calculate them. Each field in 2015 and 2016, each grassland and all the woodlands were considered as a population as all the tents for the genetic differentiation analysis.

*Tableau 2.1: Characteristics of sampled sites*

Site types	Sampling dates	Sampling modes	Locations	Number of sampling points	Number of genotyped individuals
OSR crops	June 2015	Tent	4 Fields (1- 4)	16	621
Woodland	Feb-March 2016	Tent	Several woods	53	129
Grassland	April 2016	Beating	6 Grasslands (A-G)	35	172
OSR crops	April 2016	Beating	5 Fields	50	360

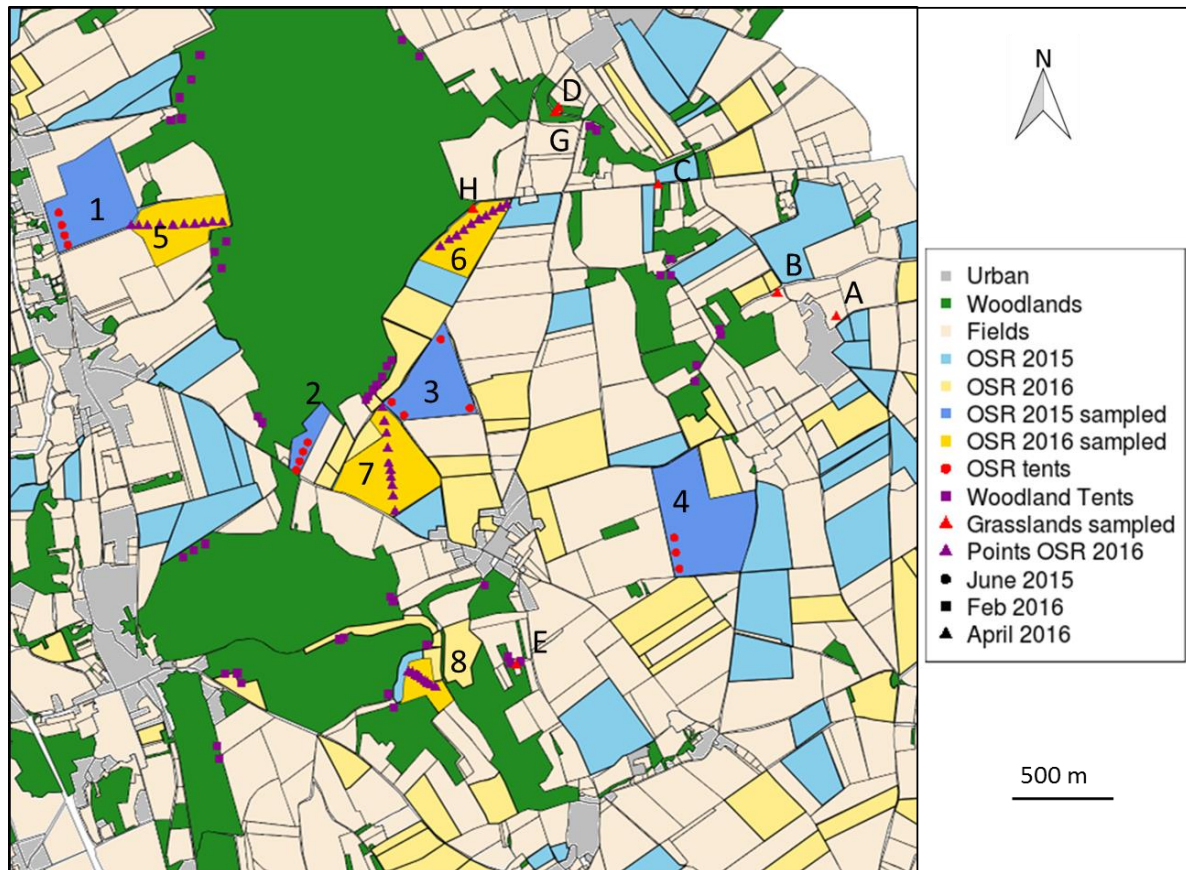


Figure 2.1: Map of the study sites in Normandy, France including the sampling points. Blue field: OSR 2015, Dark blue fields (1-4): OSR 2015 sampled. Yellow field: OSR 2016, Dark yellow fields (5-8): OSR 2016 sampled. Green fields: woodlands. Purple squares: woodland tents. Red points: OSR tents. Red triangles: grassland and roadside sampling points. Purple triangles: OSR sampling points. A-G: grasslands sampled

## 2.2. Microsatellite analysis

Thirteen microsatellite loci (*Ma-C4QRG*, *Ma-CEALQ*, *Ma-CVT0N*, *Ma-EL7YR*, *Ma-EB7XX*, *Ma-D3QFM*, *Ma-DCH30*, *Ma-DDEYS*, *Ma-DM3QY*, *Ma-DQM5T*, *Ma-EPL2N*, *Ma-ESPVQ*, *Ma-ESVIZ*, Juhel et al. 2017c) were scored for a total of 1,397 adults. We extracted the DNA by grounding each individual in 50  $\mu$ l of water using 2 mm steel beads on a 1600 MiniG (Spex® SamplePrep) tissue homogenizer at 1500 strokes/min for 30 seconds. At each sample were added 50  $\mu$ l of 20% Chelex 100 (Biorad) solution and 6% of 10 mg/ml proteinase K (Eurobio). Tissues were digested 14 hours at 56°C Mastercycler thermocycler (Eppendorf) with a final temperature step of 30 minutes at 98°C, supernatant was used as DNA template for PCR reaction. PCR amplifications were carried out with a Mastercycler thermocycler (Eppendorf) in a 10  $\mu$ l reaction volume containing 5  $\mu$ l of master-mix (QIAGEN), 2  $\mu$ l of primer-mix (primers concentration ranging from 0.13 to 2.5  $\mu$ M), and 2  $\mu$ l of DNA template. The PCR conditions were: 15 minutes at 95°C followed by 35 cycles at 94°C for 30s, 54°C for 90s, and 72°C of elongation for 1 min with a final extension step at 72°C for 20 min. Each PCR multiplex

products was diluted in 40  $\mu$ l H<sub>2</sub>O. 2 $\mu$ l of this dilution with 7.8  $\mu$ l of HiDi formamide, and 0.2  $\mu$ l GeneScan™- 600 LIZ® Size standard (Applied Biosystems) was injected on an ABI 3730xl DNA Analyzer (Applied Biosystems) using POP7 polymer (Applied Biosystems). Genotypes were visualized using GeneMapper®, version 4.1 (Applied Biosystems).

### **2.3. Population genetic variability**

Basic statistics for microsatellite loci were performed on predefined population samples considering either the individuals sampled in one tent in OSR field, or the individual sampled at one location on the same date (OSR fields or grassland or woodland locations). We estimated heterozygote proportion, gene diversity, and proportion of null alleles at each microsatellite locus in each population sample, together with their standard deviation, presented hereafter with  $\pm$  within square brackets, using GENEPOP version 4.1 (Rousset 2008). We computed exact tests for Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD). Allelic richness in each population sample was estimated using a rarefaction method (Hurlbert, 1971), using the HP-RARE program (Kalinowski, 2005) parameterized with the smallest population sample size.

### **2.4. Sibship assignment**

Sibship analysis was conducted using COLONY version 2.0.6.3 (Jones and Wang 2010). Sibship assignments were based on likelihood ratio tests assuming pollen beetle of both sexes are polygamous and using the Full-Likelihood and Pair-Likelihood score combined method (FPLS). Sibship assignment based on the FPLS method is more accurate in the detection of sibs than the pairwise likelihood method and is computationally much more efficient than the full-likelihood method (Wang, 2012).

Previous estimate of null allele frequencies (Juhel et al., 2017), were used to set up allelic dropout error at each locus, ranged from 0 to 0.09. Other genotyping error rate was set at 0.001 for all the loci. We did not update the allele frequency and did not used the sibship scaling. All other parameters were set as default. We ran the model 10 times with a different seed and considered the full sib and half sib dyad that came out in all of the 10 replicates to get a more accurate assignment.

We ran these models on groups sampled at the same period : 1) all the individuals sampled in OSR in June 2015, 2) all the individuals collected in the woodland in February to April 2016, 3) all the individuals collected in the grassland or in roadsides in April 2016 4) all the

individuals collected in OSR fields in April 2016. We separated the samples from grasslands and from OSR fields even if they were sampled on the same date as these habitats provide different resources for the pollen beetles. We then ran these models splitting groups based on the location of the sampling when the number of individuals collected per location was large enough (more than 20 individuals): 1) each tent in OSR field in 2015, 2) 3 grasslands separately in April 2016, 3) each OSR field separately in 2016. Finally, to detect links between individuals from all the locations and dates, we ran another model including all the 1282 genotyped individuals.

The contemporary effective population size ( $N_e$ ) was inferred using the sibship assignment result provided by COLONY based on the estimated proportions of full-sib and half-sib dyads (Wang 2009). Among the many genetic methods available for estimating  $N_e$  (Luikart et al. 2010), the sibship assignment method was chosen because it allows population size estimation based on samples from a single cohort (Wang 2009). We estimated  $N_e$  in each of the 6 different models with the method of Wang but using only full sibs and half sibs selected in the 10 replicates.

### **2.5. *Spatial genetic structure***

We analyzed how kinship between individuals was spatially structured. We first computed the ratio of siblings over sampled pairs of individuals by bins of distance of 250 m. We tested the impact of the distance on the ratio of siblings in the pairs using a binomial general model. In addition we used semi-variograms (Wagner et al., 2005) as implemented in the package *ggene* (Rossi, 2016) to summarize the spatial genetic structure in the population given the coordinates of the sampled individuals.

## **3. Results**

We sampled a total of 7 458 pollen beetles in all our sample sites. We genotyped 621 individuals out of the 1 312 pollen beetles in OSR fields in 2015, all the 129 individuals collected in the woodlands, all the 172 individuals collected in the grasslands and roadsides and 360 individuals out of the 5816 caught by beating in the OSR in 2016.

### **3.1. *Genetic diversity***

The mean number of alleles ranged from 4.54 to 7.69 (mean = 6.08 [ $\pm 0.89$ ]), the gene diversity ( $H_E$ ) ranged from 0.56 to 0.63 (mean = 0.60 [ $\pm 0.02$ ]) and proportion of heterozygotes ( $H_o$ ) ranged from 0.58 to 0.64 (mean = 0.61 [ $\pm 0.01$ ]). The mean allelic richness was of 4.24 [ $\pm 0.15$ ].

The proportions of null allele did not exceed 0.09 (mean = 0.03). HWE was rejected for 7 of our 26 populations or sub-populations (3 OSR tents from the field 1, OSR fields 1, 3 and 4 and grassland 3). Significant LD were detected for 4 dyads of microsatellite loci ( $p > 0.000024$ ).

### 3.2. *Sibship assignments and effective population size*

We ran with COLONY the model on the 18 different data sets on diverse levels: spatial and temporal (Table 2). The model was performed 10 times for each dataset but with a different seed. Here we present only the full sibs and half sib dyads that came out in the 10 repetitions to increase confidence in our assignments. The percentage of these full sibs and half sibs dyads compared to all the dyads found in the 10 replicate was low (Table 2).

#### 3.2.1. *Temporal analysis*

The percentage of full sib dyads found in each of the four temporal models was low ( $< 0.007\%$ ). Surprisingly, the higher percentage of full sib dyads was found for the samples collected in grasslands. The effective population size  $N_e$  decreased with time.  $N_e$  estimates in OSR were about twice lower in 2016 than in 2015.

#### 3.2.2. *Spatial analysis*

At the same date, the percentages of full sib and half sib dyads were highly variable between locations. There was no significant differences between tents of the same field of OSR in 2015. The level of sibship was low given that the larvae were on a few neighbor plants suggesting that the females spread their eggs over multiple plants. The estimated effective population size was similar between tents (Tableau 2.2).

In each of the 4 OSR fields sampled in 2015, the percentage of full siblings was close to zero. The same result was found for the half sibs, except for the field 4, in which we found 6 % of half sibs. The effective population sizes were similar in all fields, but about four time lower in field four, which was the more distant to woodland.  $N_e$  between these fields was variable and decreased with the distance to the woodlands.

In the grasslands, we also found a percentage of full sibs close to zero and the percentage of half sibs varied between 2 % to 4 % with a higher value for the grassland E with 16 %.  $N_e$  were significantly different between grasslands.

No full sibs were found in the OSR fields of 2016 and a low number of half sibs, less than 1% in each of the sites. As for the other comparisons,  $N_e$  were similar between OSR fields in 2016.



## Chapitre 2 : Structuration génétique des méligèthes à une échelle locale

Tableau 2.2: Number of half sibs (HS) and full sibs (FS) dyads by models, and percentage within dyads of FS and HS possible with the by sites analysis. Ne: mean effective size [min, max]. N= sample size. Prop FS10 and prop HS10: percent of full sibs and half sibs found in the 10 repetitions compared to all the dyads found. Tents 1-4 were tents from the field 1.

Sample	Time	N	Pairwise possible	nb FS	nb HS	%FS	%HS	prop FS10	prop HS10	Ne
Temporal datasets										
OSR 2015	June 2015	621	192510	4	41	0.00	0.02	2.2	0.4	8556
Woodlands	Feb-Mar 2016	129	8256	0	11	0.00	0.13	0.0	0.5	1501
Grasslands	April 2016	172	14706	1	42	0.01	0.29	4.3	2.4	684
OSR 2016	April 2016	363	65703	0	28	0.00	0.04	0.0	0.4	4693
Spatial datasets										
Tent 1	June 2015	50	1225	1	45	0.08	3.67	4.3	7.4	53
Tent 2	June 2015	28	378	2	47	0.53	12.43	5.0	14.8	15
Tent 3	June 2015	32	496	2	68	0.40	13.71	6.7	19.4	14
Tent 4	June 2015	38	703	1	37	0.14	5.26	2.9	7.7	37
Field 1	June 2015	148	10878	2	27	0.02	0.25	3.6	1.2	750
Field 2	June 2015	220	24090	0	18	0.00	0.07	0.0	0.4	2677
Field 3	June 2015	213	22578	2	24	0.01	0.11	2.2	0.6	1737
Field 4	June 2015	40	780	1	48	0.13	6.15	3.1	8.8	32
Grassland B	April 2016	36	630	0	31	0.00	4.92	0.0	6.6	41
Grassland C	April 2016	57	1596	1	27	0.06	1.69	2.4	3.4	114
Grassland E	April 2016	31	465	3	74	0.65	15.91	5.3	18.3	12
Field 5	April 2016	75	2775	0	21	0.00	0.76	0.0	1.2	264
Field 6	April 2016	89	3916	0	10	0.00	0.26	0.0	0.6	783
Field 7	April 2016	99	4851	0	20	0.00	0.41	0.0	1.2	485
Field 8	April 2016	96	4560	0	18	0.00	0.39	0.0	1.3	507
Global model										
All samples	2015-2016	1283	822403	10	84	0.00	0.01	4.5	0.5	17498

### 3.3. Distances between sibships

A total of 94 dyads were assigned as kind (10 dyads as full sibs and 84 dyads as half sibs) in the global model performed with COLONY (Table 2). About half the full sib dyads were detected between individuals collected in the same plot at the same date (Figure 2. 2): 4 dyads were between individuals from the same tent in OSR field and one dyad was detected between individuals collected in the same grassland. Another pair was between an individual sampled in a woodland and an individual sampled in an OSR field in 2016. There were 1 660 m between these two locations. Two other dyads of full sibs were found between individuals found at 2 300 m between each other and were sampled in an OSR of 2015 and the second individuals in OSR of 2016. Finally the ultimate pair was found at 2 600 m between two individuals from tents in OSR of 2015. Three of these 10 dyads were also found in the temporal models. The other were not tested in the temporal models.



Figure 2. 2: Distribution of the full sibs found in the whole dataset.

For each date we evaluated the effect of distance on kinship. The ratio between the counts of full sibs or half sibs and the counts of sampled dyads for each distance class did not vary significantly with the distance. We did not find either any correlation between physical and genetic distance using variograms as implemented in *ggene* R package.

#### 4. Discussion

Here, we attempted to estimate the effective population size of pollen beetle populations and seasonal dispersal based on kinship assignment. The proportions of full sibs and half sibs were similar and low between sites of collection at the same date but not between sampling dates. Dispersal between dates seems to be high, at 2 kilometers.

For all the sampled populations, we found a similar genetic diversity, showing there is no bias between our populations and sites, this is in agreement with results found at the regional, national and continental scales (Juhel et al., 2017c). Sibship inferences require four major assumptions about the genetic markers that are used (Jones and Wang, 2010): no departure from the HWE in the population, unlinked and selectively neutral loci, knowledge of population allele frequencies and no genotyping errors. The set of microsatellite was developed in order to conform to these four assumptions (Juhel et al., 2017c). Nevertheless, the presence of a high level of null alleles could affect the efficiency of sibling assignments.

The number of loci and their polymorphism are significant elements in the sibship assignment analysis (Jones & Wang, 2010). Here, we had only 13 microsatellite loci but they were polymorphic with a mean number of alleles of six. A higher assignment error rate of half sibs than full sibs is known in this kind of analysis (Van Horn et al., 2008). Here to avoid this bias, we performed our models 10 times and kept only dyads selected in each of the 10 repetitions. Only a small proportion of all the dyads found were selected by the 10 models but we found the same full sibs with all models, confirming the robustness of our analysis.

In the temporal models we found few related individuals whether full sibs or half sibs. This result could be explained by the high number of individuals in the populations. Indeed, we sampled around 7 500 individuals in all our samples. In a field, there are possibly 2 million of adult pollen beetles (Juhel et al., 2017c). The possible combinations of parents are huge and could explain a very small number of full sibs and half sibling dyads. The effective population size decreased with time, except for the last date, when pollen beetles returned in the OSR fields. This decrease between OSR fields in 2015 and woodlands, perhaps we found some individuals of the same clutch. We found a lower  $N_e$  in grasslands, showing that less parents were involved in the breed for the individuals caught in grasslands. The grasslands could host the individuals from the new generation which emerge from the OSR fields of 2016 and were from the same clutch but did not disperse a lot. In these fields, the  $N_e$  was higher than in grasslands and woodlands as they were a higher number of individuals potentially descendent of a bigger set of parents.

In the spatial models, the proportion of full and half sibs was highly variable. Between tents of the same OSR field, the proportion of full sibs and half siblings dyads was similar. The effective population size was also similar between tents showing that a similar number of parents was at the origin of the sibs. The pollen beetles which emerge in the same site of oviposition have

different parents. The females seems to disperse their clutch. Pollen beetles females are known to lay their eggs in small groups of six eggs (Hopkins & Ekbom, 1999) and perhaps can lay these groups on diverse plant of the field. Disperse the clutch is common in nature to avoid kin competition (Godfray, 1992). Indeed, many studies give as the maximum number of eggs produced by a *B. aeneus* female around 100 - 300 (Blunck, 1921; Scherney, 1953, Hopkins & Ekbom 1999). If a female lay all it's eggs on the same plant, their sibs will destroy the plant and will not have sufficient resources to survive. Here we did not detect the groups of six siblings. Perhaps we did not genotyped enough individuals, or there is a high mortality rate in the ground. Some ground beetles were known to be their predators at this stage (Schlein, & Büchs, 2004).

Between OSR fields in June 2015, the proportion of full sibs and half sibs was similar except for the field 4, in which we found more sibs. The effective population size was also similar among these fields except for field 4 in which the  $N_e$  was smaller. This field was the farthest from woodlands, perhaps parents were less numerous in this fields than others, and because of that the sibs were less mixed with others. This hypothesis is probable as pollen beetles are less abundant in fields farthest from woodland than the others (Juhel et al., 2017a). This result is also confirmed by the proportion of sibs and  $N_e$  similar between OSR fields in 2016, without field far from woodlands.

Finally, two of the three grasslands, had similar proportions of half and full sibs. The last one had a higher proportion of sibs. The three grasslands had a high  $N_e$  similar to the  $N_e$  found in the OSR fields. One of the grasslands was encircled by woodlands; the emerging pollen beetles from woodlands may have been concentrated in this grassland and could stay in the grassland if there were wild brassicacea. We saw pollen beetles on *Sinapis arvensis*, a wild brassicacea and other studies confirmed we can found pollen beetles on it (Free & Williams, 1978). Another explanation could be that the individuals found in the grasslands were from the new generation which just emerged from OSR fields and have more chances to be siblings.

With the global model we found 10 dyads of full sibs distributed across time and space. These dyads were confirmed in the other models. We found dyads of full sibs in June 2015, the emerging siblings were found in the same trap but also in different fields. This could suggest that females were able to lay their eggs on different plants both within and between OSR fields. This is in agreement with the hypothesis of spread of the clutch by females. We also found one dyad within the grassland E, the same as in the local sampling, confirming the effect of woodlands as a barrier on the individuals from this grassland. Two dyads were found between

OSR fields in 2015 and OSR fields of 2016, showing that individuals can travel at least 1 km between their birth place and their breeding place. We also found a pair of full sibs between a woodland tent and an OSR field of 2016 at 1 600 m. This is in agreement with the mean dispersal distance of 1 200 m found with statistical methods (Juhel et al., 2017a) and the mean distance traveled by pollen beetles between woodlands and OSR fields by capture marking recapture of 1 000 m (Taimr et al., 1967). Nevertheless, these distances are only minimums as we did not find an effect of the distance on kinship structures, up to 4 kms.

We used sibship inference to estimate pollen beetles demographic parameters within and between diverse sites from a French department, the Eure, in a context of low population genetic structure. We showed that the proportion of full sibs and half sibs between sites is stable but can be affected by the presence or absence of woodlands. The effective population sizes were similar between sites but low, suggesting a high larvae mortality. Full sibs of one generation were found at more than 2 000 m of their birth place highlighting the difficulties to manage *B. aeneus*.

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## Chapitre 3

Caractérisation de la dispersion des  
méligèthes, *Brassicogethes aeneus*, des  
forêts vers les champs de colza





### **Chapitre 3 : Caractérisation de la dispersion des méligèthes, *Brassicogethes aeneus*, des forêts vers les champs de colza**

Dans la partie précédente, à l'aide d'analyses d'assignation de parentés, nous avons montré que les méligèthes d'une même fratrie pouvaient être observés à 2 kilomètres l'un de l'autre. Ici, nous avons cherché à évaluer la distance moyenne parcourue par les méligèthes émergeant des bois et allant coloniser les parcelles de colza sur la base d'analyses des abondances de méligèthes dans les parcelles.

Nous avons observé sur quatre dates l'abondance des méligèthes dans 24 champs disséminés dans l'Eure, en France. Nous avons modélisé l'abondance comme résultant de la dispersion depuis les forêts avoisinantes selon un kernel de dispersion. Nous avons comparé les modalités de dispersion correspondant à différentes hypothèses sur l'origine de la dispersion, la forme du kernel (Gaussien ou exponentiel) de dispersion et les sources de variabilité.

Ce travail correspond à un article accepté dans la revue *Plos One* et intitulé : “*Characterization of the pollen beetle, Brassicogethes aeneus, dispersal from woodlands to winter oilseed rape fields*”.



## **Characterization of the pollen beetle, *Brassicogethes aeneus*, dispersal from woodlands to winter oilseed rape fields**

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### **Abstract**

Many crop pests rely on resources out of crop fields; understanding how they colonize the fields is an important factor to develop integrated pest management. In particular, the time of crop colonization and damage severity might be determined by pest movements between fields and non-crop areas. Notably, the pollen beetle, *Brassicogethes aeneus*, previously named *Meligethes aeneus*, one of the most important pests of winter oilseed rape, overwinters in woodlands. As a result, its abundance increases in oilseed rape fields near wooded areas. Here, we assessed the spatio-temporal patterns of the dispersal from woodlands to oilseed rape fields in diversified landscapes of a same region. We observed on four dates the abundance of pollen beetles in 24 fields spread in the Eure department, France. We modeled the abundance as a result of the dispersal from the neighboring woodlands. We compared the modalities of dispersal corresponding to different hypotheses on the dispersal origin, kernel shape and sources of variability. Within oilseed rape the distance to the edges of woodlands is not the main determinant of pollen beetle abundance. On the contrary, the variability of the abundance between fields is largely explained by the dispersal from neighboring woodlands but there is considerable variability between dates, sites and, to a lesser extent, between fields. The two dispersal kernels received similar support from the data and lead to similar conclusions. The mean dispersal distance is 1.2 km but seems to increase from a few hundred meters the first week to more than two kilometers the fourth, allowing the pollen beetles to reach more distant OSR fields. These results suggest that early varieties away from woodlands and late varieties close to the woodlands may limit attacks at the time when oilseed rape is the most sensitive.

**Key words:** *Brassicogethes aeneus*; integrated pest management, mean dispersal distance, landscape, spatial scale

## 1. Introduction

Understanding pest dispersal is crucial for the design of integrated pest management strategies, particularly when non-crop areas are potential sources of pests that recolonize surrounding fields [1]. The pollen beetle *Brassicogethes aeneus* (Fabricius, 1775) (formerly *Meligethes aeneus*) (Coleoptera: Nitidulidae) is one of the most important insect pests in winter oilseed rape (OSR; *Brassica napus* L.) in Europe [2]. In many countries, OSR receives a large fraction of the pesticides used on grain crops [3]. As a result, insecticide resistance is widespread among OSR pests; for example, pollen beetles in Europe became generally resistant to pyrethroids [4], [5]. Alternative integrated pest management strategies for OSR crops have been actively sought for in the last decade, such as repellents [6], traps [7] or resistant cultivars [8]. At the field scale, methods such as trap crops have been suggested to limit the damage to the crop [7]. At the landscape scale, it has been suggested to improve the management of semi-natural habitat surrounding crops [9]. The life cycles of the pests or of their natural enemies often involve semi-natural habitats providing overwintering or feeding sites [10]. The pollen beetle eggs hatch in OSR buds, and the larvae develop within buds and drop to the ground to pupate. The new generation of pollen beetle emerges a few weeks later, in the early summer, and seeks overwintering sites in woodlands [11], [12]. In the early spring (March-April), the adults migrate from the woodlands to OSR fields to feed on pollen and oviposit [2]. If they arrive before flowering, they destroy the buds to feed and can inflict severe yield losses [13].

In such a life cycle, dispersal capacities play a crucial role, in particular allowing movements from woodlands to fields and from fields to woodlands [14]. Beyond studying correlations between pollen beetles abundance data and landscape elements [12], [15], [16] characterizing the dispersal could improve the prediction and management of the pollen beetle. As the timing of their arrival in the crop is decisive for the damage, understanding the temporal dynamic of their dispersal would further help the design of management strategies. Finally, considering dispersal both within and between crops would help design attraction based management strategies such as trap crops.

The dispersal distance has been studied by marking insects with radioactive elements [17], [18]. A first study found the pollen beetles to disperse in the spring (March) up to ten kilometers within two days; though ten kilometers was also the maximal distance covered in twelve days [17]. For the summer dispersal (July), a mean distance per days of 1 to 3 km was observed [18]. Dispersal distance can also be estimated fitting dispersal kernels to abundance data, which

potentially yields very different estimates [19]. Moreover, pollen beetles flight could be influenced by temperatures [16]; the height of the flight had also been studied [6]. Within a field pollen beetles are more abundant at the edges but little is known on their dispersal in this spatial scale [2], [20], [21] .

Here we investigated the mean dispersal distance covered by adult pollen beetles from woodland to OSR fields during spring. We sampled adults in 24 OSR fields over a broad landscape and fit alternative models to these data to characterize the dispersal. Specifically we investigated whether the average dispersal distance changes with time or with the complexity of the landscape. We also tested if the specific location of the points in the fields was relevant or if considering all sampling in a field to be at the same median place was more adequate, suggesting a different type of dispersal within OSR fields. Finally, we investigated if the abundance in the field was best explained by considering the whole area of large woodlands as a source of pollen beetle or by considering only the first 100 m within the woodland from their edges as a source of pollen beetle.

## **2. Material and methods**

### ***2.1. Study site***

The study was carried out in 2015, in four agricultural landscapes from the Normandy region in north western France bounded at North West at 49°00'31.9"N 1°10'23.0"E, at South West at 48°51'47.8"N 1°12'51.5"E, at 48°56'56.5"N 1°30'15.6"E for South East and at 49°00'08.6"N 1°27'42.6"E for North East (Fig 1).

This area has a sub-oceanic temperate climate, with mild summers (mean monthly temperature from May to July in 2015, 20.9 [10.7-23.8] °C; monthly mean precipitation: 53.2 mm) and cool late winters (mean monthly temperature for January and February 2015: 4.1 [1.2-7.1] °C; mean monthly precipitation: 45.9 mm). The semi-natural habitat consists in large woodland areas and small woodlots, hedgerows and grasslands; all of which have been related to the life cycle of the pollen beetles [2]. The OSR is cultivated in relatively high proportion of the cultivated fields (Fig 1). We defined four sites of about four by six kilometers with a growing proportion of woodlands. Note that in addition to the growing proportion of woodlands, the first two were on a plateau with little to no hedgerows, while the two others were centered on valleys with a hedged landscape (as qualitatively assessed by visual inspection of the sites) and also with more grasslands. This growing landscape complexity prompted us to label these landscapes by their complexity as Highly Simple: HS, Simple: S, Complex: C, Highly Complex: HC (Fig 1).

## ***2.2. Insects sampling***

We selected 24 OSR fields distributed over the four sites (four to seven fields per site, Figure 3.1). Nine fields, among the 24 selected fields, were chosen aligned along three transects in two sites (Fig 1) from important woodlands in the area ( $> 1 \text{ km}^2$ ), allowing visualization of the gradient of abundance when getting further from these woodlands. In each of the 24 selected fields we sampled five aligned points approximately separated by 50 m. These transects moved from the edge of the field toward the middle of the field perpendicularly to its edge. In two fields at immediate proximity of a major woodland (red circles Fig 1), in the field, we sampled five additional points allowing to visualize a potential gradient of abundance within a field.

At each sampling point, we sampled adult pollen beetle by beating ten plants inside plastic bags. These ten plants were chosen at 1 m from each other to avoid pollen beetles falling from crops while beating nearby. We repeated the sampling four times, one week apart between March 25<sup>th</sup>, 2015 and April 16<sup>th</sup>, 2015. The OSR shifted during this period from bud developments stage (Growth Stage GS 50) to the end of the flowering stage (GS 65). Among the 3657 pollen beetles captured, 230 individuals equally distributed among the four study sites were identified by their meta-femur [22]. Most pollen beetle were identified as *B. aeneus* (99%). Only two specimens were identified as *B. viridescens* (Fabricius, 1787). Consequently, we did not differentiate the two species in the following analysis of the abundance. For all our analyses, we defined the abundance of pollen beetles as the number of pollen beetles for ten OSR plants, i.e. the number of pollen beetles caught at a given sampling point and date.

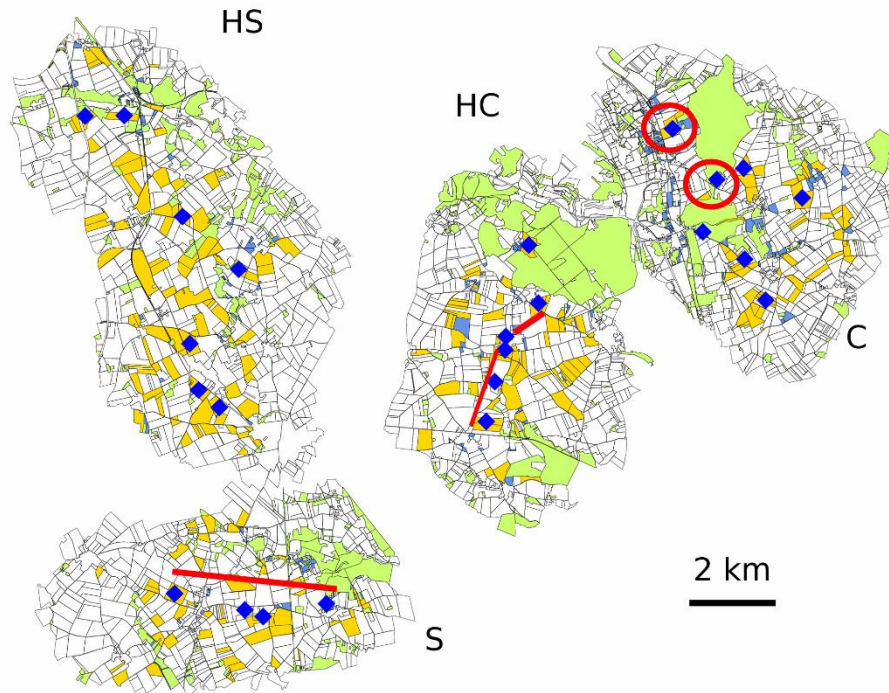


Figure 3.1 Map of the four studied sites (Normandy region, France), describing land uses within two kilometers radius buffer around the barycenter of the sampled OSR fields in 2015. Green: woodlands. Blue: grasslands. Yellow: OSR crops. Diamond: sampled OSR fields. Red lines: between fields transect lines. Red circles single out fields where we followed within-field transects from woodlands over 200 m. We distinguish four sites with differing complexities: Highly Simple: HS (7 fields), Simple: S (4 fields), Complex: C (7 fields), Highly Complex: HC (5 fields). Complexity here is a gradient of woodland surfaces in each sites, simple is a site with few woodlands.

### 2.3. Mapping and geomatics

We mapped forests and field delimitations respectively within five or two kilometers from sampled fields using aerial photographs (GoogleMyMaps pixel size: 0.5 m, 2017 TerraMetrics). To help discussion of the results, we also mapped grasslands and OSR fields within two kilometers of the selected OSR fields (Fig 1). Hedgerows were not mapped as they were more difficult to identify from aerial photographs. To account for wooded areas in the models, we discretized the woodlands on a grid of 100 m resolution (pixel of 100 m\*100 m) and affected to each pixel the wooded area it covered. Previous studies did not find differences in emergence abundance after overwintering within the first 100 m within the woodland from their edges than deeper in the woodland [11]. To assess if more central parts of the woodland are less important hibernating sites than the periphery, we here differentiate the 100 m wide periphery from the more central parts of the woodlands.



We calculated woodland areas and distances of each sampling point to woodlands using the R package `rgeos` [23] using Universal Transverse Mercator (UTM) projection system. All geomatics treatment and statistical analyses were performed using R software (R Core Team 2016).

## 2.4. Statistical analysis

We modeled the abundance (total number) of pollen beetle at each sampled point as a random variable in a Bayesian perspective. Abundance data are commonly described using a Poisson distribution; here, as the data tended to be over-dispersed we assumed the abundance to follow a negative binomial distribution of mean  $\mu$  varying by date  $t$  and sampling point  $i$ . The size parameter for this distribution, strictly positive, was fitted using a flat prior on the log scale.

### 2.4.1. Modeling the mean abundance of pollen beetle

We modeled the expected abundance  $\mu$  observed at a given time as the product of the intensity  $\gamma_e$  of pollen beetle arriving at the sampling point and a categorical size factor  $\gamma_g$ :

$$\mu = \gamma_e \cdot \gamma_g \quad (1)$$

The size factor  $\gamma_g$  was a catch-all categorical parameter allowing to model different sources of variability. The visibility of the pollen beetle in the crop is highly dependent on the weather [13], in addition the attractiveness of the crop for the pollen beetle varies with time [24], so each date is considered separately in  $\gamma_g$ . We also considered the possibility to have a specific category per location (at field or site level). Finally, we considered the possibility that a different category should be fitted for each location-date couple. For this categorical size factor  $\gamma_g$  we used as a non-informative prior a Cauchy distribution with center 0 and scale 2.5 [25].

The pollen beetle arriving at a sampling point  $i$  was the sum of the pollen beetle arriving at date  $t$  from each surrounding unit of woodland  $w$  of area  $A_w$  within 5 km. The expected number of pollen beetle  $\mu(t,i)$  observed in field  $i$  becomes:

$$\mu(t, i) = \gamma_g \sum_w \gamma_d(i, w, t) \cdot A_w \quad (2)$$

Where  $\gamma_d(i,w,t)$  was a dispersal kernel from the unit of woodland  $w$  to a field  $i$  at date  $t$ . This representation does not imply that the flight is direct from the source to the destination, on the contrary, different kernels of dispersal can be interpreted as different types of path, with more or less relay habitats and more or less random or directed movement to the destination.

### 2.4.2. Dispersal kernels

We tested two commonly used dispersal kernels: the Gaussian kernel and the Exponential kernel [26]. These two contrasted kernels are chosen to make sure the results are not too dependent on the shape of the kernel. These kernels were considered to be a function of the date  $t$ :

$$\gamma_d(i, w, t) = \frac{1}{K\pi\delta(t)^2} \exp\left(-\left(\frac{d_{iw}}{\delta(t)}\right)^n\right) \quad (3)$$

Where  $K$  and  $n$  varied with the dispersal kernels (Gaussian kernel:  $K=1$  and  $n=2$ , exponential kernel:  $K=2$  and  $n=1$ ).  $d_{iw}$  was the distance from the woodland unit to the sampled point.  $\delta(t)$  is a scale parameter, homogenous to a distance and a function of time. To test if the dispersal varies with time, we set that the abundance decreases with the distance ( $1/\delta(t)$ ) and changes linearly with time by a factor  $\beta_{dt}$  each week from an intercept  $\beta_d$  at the first date ( $t=1$ ) as follow:

$$\delta(t) = 1/(\beta_d + \beta_{dt} (t - 1)) \quad (4)$$

For the dispersion parameters  $\beta_{dt}$  and  $\beta_d$  we applied a flat prior on their definition domain. Their definition domain corresponds to a positive distance of dispersion:  $\beta_d > 0$  and  $\forall t \in \{1, 2, 3, 4\} \beta_d + \beta_{dt} (t-1) > 0$ .

For an exponential kernel, the mean dispersal distance  $D_{\text{mean}}$  is given by:

$$D_{\text{mean}}(t) = \frac{2}{(\beta_d + \beta_{dt}(t-1))} \quad (5)$$

For a Gaussian kernel,  $D_{\text{mean}}$  is given by:

$$D_{\text{mean}}(t) = \frac{\sqrt{\pi}}{2 \cdot (\beta_d + \beta_{dt}(t-1))} \quad (6)$$

### 2.4.3. Fit and comparison of models

We compared the fit of the models using the total area of the woodlands or just the 100 m peripheral area to test if pollen beetle can be considered to only emerge from the 100 m peripheral area. To evaluate the effect of distances from woodlands within fields, we compared a model considering the actual coordinates of the five points in a field (split) and a model considering the five samples to be at the barycenter of the points (merged). In the second model the position information within the fields is hidden, preventing the fit of a potential gradient within fields. Comparing the two models allows then to say if considering a gradient within the

field improves the fit and hence if the gradient from woods levels off or not within fields. We also considered a model without geographical coordinates (“none”) to assess the relevance of accounting for the proximity of woodlands. Finally, to test the effect of landscape complexity on the dispersal, we tested if fitting the parameters  $\beta_d$  (factor determining the average dispersal distance from woodlands to OSR) and  $\beta_{dt}$  (factor modifying the average dispersal distance with time from woodlands to OSR with time) separately for the four sites that had different complexities improved the adjustment to the data. Testing different possible categorical factors to describe the location and date allowed to check the robustness of the results to the above tests. Combining these modalities (Tableau 3.1) led to test 161 models. .

Each of these models was adjusted using a Monte Carlo Markov chains (MCMC). Each MCMC was run until the Geweke diagnostic [27] and the Raftery and Lewis diagnostic [28] were satisfied as implemented in the YAMH, R package [29].

The confidence intervals for the estimates correspond to the quantiles 2.5 % and 97.5 % of the values sampled in the Markov chains. Hereafter the parameter values we present correspond to the set maximizing the likelihood in the Markov chain unless the likelihood corresponding to the median value in the Markov chain for each parameter provides a higher likelihood. We then ranked all the models by their AICs (30).

## Chapitre 3 : Caractérisation de la dispersion des méligèthes

Tableau 3.1: Parameters and modalities used to test the 161 models

Parameter	Modalities	Description and hypothesis	Definition domain
$\beta_d$	-	<b>Dispersal distance factor</b>	$]0; +\infty[$
$\beta_{dt}$	-	<b>Modification of the dispersal distance factor by time</b>	$\beta_d + \beta_{dt} (t-1) > 0$
<b>Size factor</b>		<b>Date and location categorical effect</b>	
	Date	Effect per date of sampling	$]-\infty, +\infty[$
	Field	Effect per field	$]-\infty, +\infty[$
	Site	Effect per sampling site	$]-\infty, +\infty[$
	Field + date	Separate field and sampling date effects	$]-\infty, +\infty[$
	Site + date	Separate site and sampling date effects	$]-\infty, +\infty[$
	Field : date	An effect per field-date couple	$]-\infty, +\infty[$
	Site : date	An effect per field-date couple	$]-\infty, +\infty[$
	"1"	Same factor for all sampling points and dates	$]-\infty, +\infty[$
<b>Origin</b>		<b>Part of woods considered as a source of <i>B. aeneus</i></b>	
	Area	Total woodland area	-
	Edge	Edges of woodland area (first 100 m within woods)	-
<b>Field point structure</b>		<b>Coordinates of the sampling points used in the model</b>	
	Split	Actual coordinates of the five points in a field	-
	Merged	The barycenter of all the points sampled in the field	-
	None	Coordinates not accounted for (no distance effect)	-
<b>Kernel</b>		<b>Shape of the dispersal kernel</b>	
	Exponential	Exponential decrease from woodlands (oriented dispersal)	-
	Gaussian	Normal decrease from woodlands (random dispersal)	-
<b>Site dependent dispersal</b>		<b><math>\beta_{dt}</math> and <math>\beta_d</math> fitted separately in each site ?</b>	
	True	Dispersal varies by site (landscape complexity)	-
	False	Same dispersal in all sites (landscape complexity)	-

2.4.4. *Relating pollen beetle abundance to woodland areas in buffers.*

In a similar way to previously published work [12], [14], [31], we also modeled the pollen beetle count data as a function of woodland areas in buffers around the sampling points:

$$\mu(t, i) = \gamma_g A_{BW}$$

With  $A_{BW}$  the area of woodlands in a given buffer (disk) around the sampling point. We used a glm assuming a negative binomial distribution of the pollen beetle count data, with the canonical log link function. The above becoming:

$$\log(\mu(t, i)) = \beta_G + \log(A_{BW})$$

Where  $\beta_G$  corresponds to the parameters for the categorical factors G factors for time and localization as described for the kernel based models. We fitted the buffer radius by selecting the model with the best likelihood for models with radii ranging from 0.2 to 5 km by increments of 200 m.

### 3. Results

The mapping of the four sites allowed to rank the four sites from highly simple (HS) to highly complex (HC) (Tableau 3.2).

Tableau 3.2: *Landscape characteristics of the four study sites in 2009 (within two kilometers of sampled fields). Woodland edges are the first 100 m within the woodlands from its edge.*

Site name	Area (km <sup>2</sup> )	size Woodland core	Woodland edges	Grassland	OSR crops	Other crops
Highly Simple (HS)	56.9	7.4%	6.8%	0.8%	14.8%	66.7%
Simple (S)	31.5	13.9%	9.9%	0.7%	8.4%	65.3%
Complex (C)	47.1	18.2%	10.9%	3.0%	8.4%	58.1%
Highly Complex (HC)	35.8	22.7%	10.4%	1.2%	9.9%	54.4%

Area size (km<sup>2</sup>), proportion (%) of woodlands edges and core, grasslands, OSR and other crops in each site.

Abundance of pollen beetle was low but compared to other observations in Europe [15] but highly variable between sites, fields and even within fields (Tableau 3.3). The average pollen beetle abundance was higher in more complex sites and reached its peak on April 8<sup>th</sup> ( $t_3$ ) (Tableau 3.3).

Tableau 3.3: Observed pollen beetles mean abundance [ $\pm$ SE] (number for ten plants) by site and date.

Site	t <sub>1</sub> : March 25 <sup>th</sup>	t <sub>2</sub> :April 1 <sup>st</sup>	t <sub>3</sub> :April 8 <sup>th</sup>	t <sub>4</sub> : April 15 <sup>th</sup>	All
HS	2.4 ( $\pm$ 5.4)	0.3 ( $\pm$ 0.8)	3.9 ( $\pm$ 4.8)	1.6 ( $\pm$ 1.8)	2.6 ( $\pm$ 3.9)
S	1.3 ( $\pm$ 3.7)	0.4 ( $\pm$ 0.9)	6.4 ( $\pm$ 6.4)	2.9 ( $\pm$ 2.6)	2.7 ( $\pm$ 3.3)
C	2.5 ( $\pm$ 3.7)	2.5 ( $\pm$ 2.9)	33.8 ( $\pm$ 26.2)	12.6 ( $\pm$ 12.8)	13.2 ( $\pm$ 17.6)
HC	1.3 ( $\pm$ 2.2)	0.5 ( $\pm$ 0.9)	16.1 ( $\pm$ 21.0)	7.2 ( $\pm$ 7.8)	6.5 ( $\pm$ 11.7)
All	3.8 ( $\pm$ 4.3)	0.9 ( $\pm$ 1.5)	15.2 ( $\pm$ 20.5)	6.7 ( $\pm$ 9.1)	

Mean values ( $\pm$ SD)

### 3.1. Visible influence of the distance from the main woods at the kilometer-scale

The abundance easily reaches 5-fold variations in a given field at a given date. Within field abundance were highly variable and in a case the highest abundance in a field was reached in the middle of the field (Figure 3.2A). On the contrary, the abundance of pollen beetles merged by field show a clear and quick decrease of pollen beetles abundance with the distance from woodlands over a few kilometers (Figure 3.2B). The impact of the date is also major with very few pollen beetle captured on the date 2.

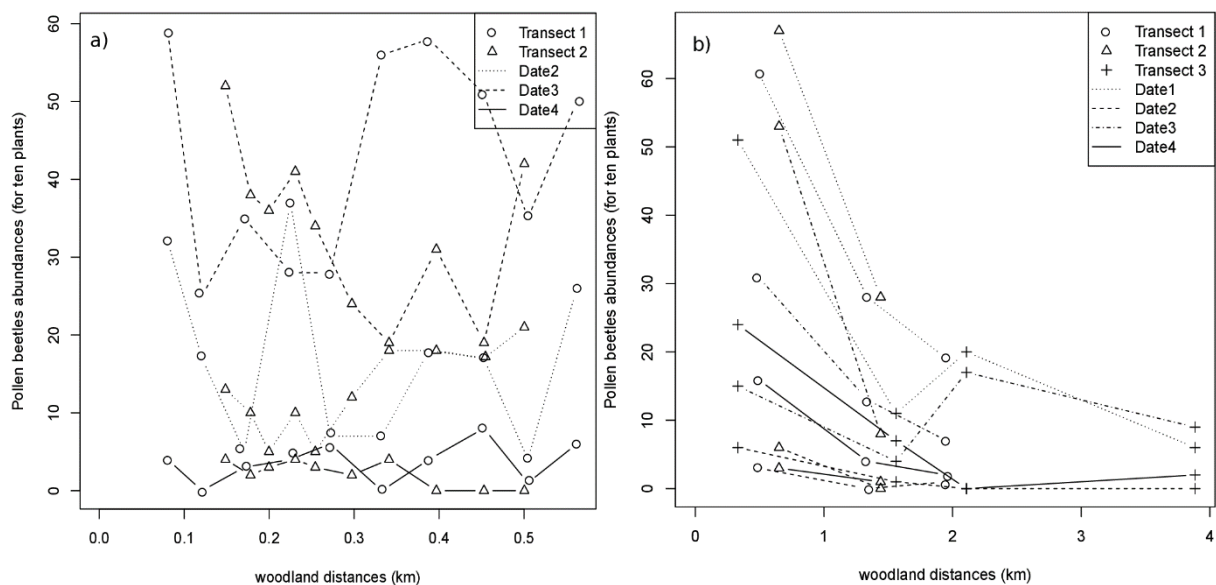


Figure 3.2 Variation of pollen beetle abundance along within-field (A) and between-field (B) transects as a function of distance (km) to woodland edges. (A) Number of pollen beetles per sampling point within two different OSR fields (fields in complex site, C). (B) Mean number of pollen beetles per field in three transects (in zones S, for simple landscape, and HC, for highly complex landscape). Abundance were measured on 10 OSR plants at each sampling point during three (A) or four (B) consecutive weeks (dates 1 to 4). Trans: Transect

### ***3.2. Significant effect of the date, the field and dispersal from woods***

Comparing the AIC of the 161 models, allowed us to identify the dispersal modalities best explaining the observed patterns (Table 4), all models are presented in S1 Table and Data are available in the S2 Table. The best model (Table 4, N°1) accounted for the effect of fields and the effect of the date separately, it kept the spatial structure of the points within a field (split), used a Gaussian kernel and the whole area of the woods was considered as a source of pollen beetle. Finally, the dispersal parameters were fitted separately for each site (Tableau 3.4).

Removing the date size effect had the strongest impact on the AIC: the models without date effect constitute the top were the worst, several hundred points of AIC behind the best. Including a location effect, at least at site level was also important as it allowed to get within a hundred AIC points of the best model. Allowing an interaction between site and date effect further improved the description of the data by 20 points of AIC. Using date and field effect separately had a clear impact with a difference of 5 points of AIC.

Constraining the dispersal still had a strong impact ( $\Delta$  AIC > 10 points) whether the dispersal was forced to be homogeneous between sites, the points within a field were merged or no effect of the distance to the woodlands were considered. Only considering the 100 m periphery of the woodlands as sources had a limited impact on the fit (AIC variation of seven points and similar estimates). Finally, the best model retained a Gaussian kernel but imposing an exponential kernel had a limited impact on the fit (AIC variation of four points) and yield similar mean dispersal distances and impact of time on the dispersal (Tableau 3.4).

### ***3.3. The field effect obfuscates the dispersal from woodlands in complex sites***

Measuring the importance of the woodlands by differentiating the best model (Table 3.4, N°1) with the best model without woodlands (Table 3.4, N°4) can minimize the effect of woodlands as the variability otherwise explained by the woods is transferred to the field effect. Using as reference the best model without field effect (Table 3.4, N°7), the  $\Delta$  AIC, removing the effect of wood (S3.1 Table, N°87), attained 180 confirming the importance of the woodlands.

### Chapitre 3 : Caractérisation de la dispersion des méligèthes

Tableau 3.4: Best models obtained out of the 161 models estimating pollen beetles dispersal from woodlands to OSR, constraining in turn each modality (categorical factor type, origin, Field point structure, kernel and site dependant dispersal) leaving other modalities and parameters free, with or without a field effect. **Bold**: parameter constrained, allowing a field effect. Underlined: parameter constrained, not allowing a field effect.

N°	Categorical factor type	Origin	Same field point structure	Kernel	Site dependent dispersal	AIC	$\Delta$ AIC	Mean $\beta_{dt}$	Mean $D_{mean}$ (km)
1	<b>Date+field</b>	<b>area</b>	<b>split</b>	<b>Gaus.</b>	<b>TRUE</b>	2419.8	0	0.22 [0.07,0.38]	3.4 [1.6,5]
2	Date+field	area	split	<b>exp.</b>	TRUE	2424.0	4.2	0.57 [0.18,1.07]	4.2 [2.8,6.5]
3	Date+field	<b>edge</b>	split	Gaus.	TRUE	2427.7	7.9	0.2 [0.05,0.38]	3.1 [2.4,4.1]
4	<b>Date:field</b>	<b>NA</b>	<b>none</b>	NA	NA	2433.7	13.9	NA	NA
5	Date+field	area	<b>merged</b>	exp.	TRUE	2435.4	15.6	0.52 [-0.05,1.4]	4.1 [3.0,6.6]
6	Date:field	area	merged	Gaus.	<b>FALSE</b>	2440.1	20.3	0.03 [-0.08,0.16]	2.2 [1.5,3.0]
7	<u><b>Date:site</b></u>	<u>edge</u>	<u>split</u>	<u>Gaus.</u>	<u>TRUE</u>	2453.3	33.4	0.26 [0.1,0.42]	1.2 [1.0,2.6]
8	Date:site	edge	<u>merged</u>	Gaus.	TRUE	2454.6	34.8	0.16 [0.04,0.29]	1.2 [1.0,1.7]
9	Date:site	edge	merged	Gaus.	<u>FALSE</u>	2454.9	35.1	0.07 [-0.03,0.18]	1.1 [1.0,1.3]
10	Date:site	edge	split	<u>exp.</u>	TRUE	2457.8	38.0	0.61 [0.18,1.06]	1.3 [1.3,1]
11	Date:site	<u>area</u>	split	exp.	TRUE	2466.2	46.4	0.54 [0.14,0.95]	1.8 [1.3,3.0]
12	<u><b>Date+site</b></u>	edge	merged	Gaus.	TRUE	2474.0	54.1	0.03 [-0.08,0.21]	2.4 [1.5,2.8]
13	<b>Date</b>	edge	merged	Gaus.	FALSE	2568.3	148.4	0.09 [-0.02,0.18]	1.1 [0.9,1.3]
14	<u><b>"1"</b></u>	area	split	exp.	TRUE	2737.4	317.6	1.48 [0.68,2.29]	2.6 [1.4,5.6]
15	<u><b>field</b></u>	edge	split	Gaus.	TRUE	2770.4	350.5	0.19 [0.03,0.51]	4.1 [2.0,6.2]
16	<u><b>site</b></u>	area	none	Gaus.	FALSE	2934.9	515.1	NA	NA

Factor type: type of factor. Origin: whole wood area or only 100 m wide area bordering woodland area. Same field point structure: split = with real coordinates of points. Same field point structure = all points of a field with coordinates of barycenter, none = no coordinates. Kernel = Exponential (exp.) or Gaussian (Gaus.) dispersal kernels. Site dependent dispersal: with or without interaction between site and distance.  $\Delta$  AIC : difference of AIC with first model. Mean  $\beta_{dt}$ : mean effect of time on the dispersal distance. Mean  $D_{mean}$ : mean dispersal distance from woodlands to OSR.

Using as a reference the best model without field effect also changed the relevance of some dispersal modalities (Table 3.4 N°7). Most notably, edges became more relevant than the whole area (13 points of  $\Delta$  AIC when it was 7.9 points of  $\Delta$  AIC the other way around). Using real coordinates (split) was only marginally better than merging them suggesting that the reality might be between the two contrasted hypotheses represented by these models: the gradient within fields would be neither flat nor as strong as between fields. Finally, estimating the dispersal separately for each site was only marginally better than using the same dispersal parameters for all the sites. The Gaussian kernel still better represented the abundance though the use of the exponential kernel did not change the estimated dispersal characteristics.



**3.4. The dispersal is similar in simple and complex landscapes**

The best model included an effect of distance from the woods (point structure different from none, Tableau 3.5 N°1). The estimation of the mean dispersal distance  $D_{\text{mean}}$  was similar in the two simple landscapes (1.46 and 1.01 km, Tableau 3.5). In the two more complex landscape,  $D_{\text{mean}}$  tended to be very high at least for the first sampling dates (Tableau 3.5). Given that we only accounted for woods within five kilometers would be that the proximity from woodlands did not impact the abundance in complex landscapes, a surprising conclusion when looking at the abundance as a function of the distance from the major woodlands (Figure 3.2 B, Transect 1 and 2).

Tableau 3.5 : Value of fitted dispersal distance global ( $D_{\text{mean}}$ ) at each date ( $D_{\text{mean}}(t_n)$ ) and impact of time on the dispersal ( $\beta_{dt}$ ) for the best model (N°1) and for the best model without field effect (N°7).

Sites	$D_{\text{mean}}(t_1)$	$D_{\text{mean}}(t_2)$	$D_{\text{mean}}(t_3)$	$D_{\text{mean}}(t_4)$	$D_{\text{mean}}$	$\beta_{dt}$
<b>Best model with fields (N°1)</b>						
HS	0.6 [0.4,1.2]	0.84 [0.5,1.36]	1.3 [0.8,2.0]	3.0 [1.1,5.4]	1.5 [0.7,2.2]	0.4 [0.2,0.7]
S	0.4 [0.2,0.8]	0.48 [0.3,0.98]	0.8 [0.5,1.4]	2.2 [0.7,5.5]	1.0 [0.5,1.8]	0.7 [0.2,1.3]
C	10.5 [1.2,13.8]	3.62 [1.3,4.93]	2.2 [1.3,3.2]	1.6 [1.0,2.3]	4.5 [1.4,5.7]	-0.2 [-0.2,0.0]
HC	11.7 [1.5,21.1]	6.63 [1.65,13]	4.7 [1.8,9.5]	3.6 [1.8,7.5]	6.6 [1.8,12.7]	-0.1 [-0.1,-0.0]
<b>Best model without fields (N°7)</b>						
HS	0.5 [0.3,0.8]	0.7 [0.5,1.0]	1.0 [0.7,1.5]	2.3 [1.1,13.3]	1.2 [0.8,3.9]	0.5 [0.2,0.8]
S	0.4 [0.2,0.8]	0.5 [0.3,1.0]	0.8 [0.6,1.4]	2.6 [1.1,2.5]	1.1 [0.7,5.4]	0.7 [0.2,1.2]
C	1.2 [0.7,2.3]	1.1 [0.8,1.6]	1.1 [0.8,1.3]	1.0 [0.8,1.5]	1.1 [0.9,1.5]	-0.1 [-0.2,0.1]
HC	1.6 [1.0,3.3]	1.4 [1.02,1.9]	1.2 [1.0,1.5]	1.1 [0.9,1.5]	1.3 [1.0,1.9]	-0.1 [-0.2,0.2]

Model N°1: Time + fields effects, spatial structure of the points within a field, Gaussian kernel, whole area of the woods, dispersal parameters fitted separately for each site.

To test if the effect of distance from the woodlands might be hidden by the field effect in more complex landscapes, we looked at the average distances fitted for the best model without field effect (Table 5, N°7). Not only fitting the dispersal separately for the four sites improved the AIC only marginally (Table 5, N°7 vs. 9), but the fitted distances were similar in the four sites and also similar to the distances fitted with the field effect in the simple landscape sites (1.2 km) (Table 5). This suggests that the dispersal was similar in simple and complex landscapes though the field effect tended to hide it (Table 5). When a real impact of the distance to the woods is detected, the average distance traveled by the pollen beetles was close to 1.2 km. The Figure 3. 3 allows to appreciate the correspondence between calculated and observed cumulative abundance per field over the four dates for this model without field effect and the

strong relationship between the abundance and the proximity to woodlands (Fig 3). Over the four sites, the dispersal distance also tended to increase with time as the mean  $\beta_{dt}$  was significantly positive for the best models both with field or site effect, when not estimating separately the dispersal parameters per site. The corresponding partial fading out of the gradient from major woods can be visualized in Figure 3.2B) and the effect ( $\beta_{dt}$  confidence interval not including zero) was particularly clear in simple landscapes (Table 5), suggesting that the pattern might be similar though obfuscated in complex landscapes.

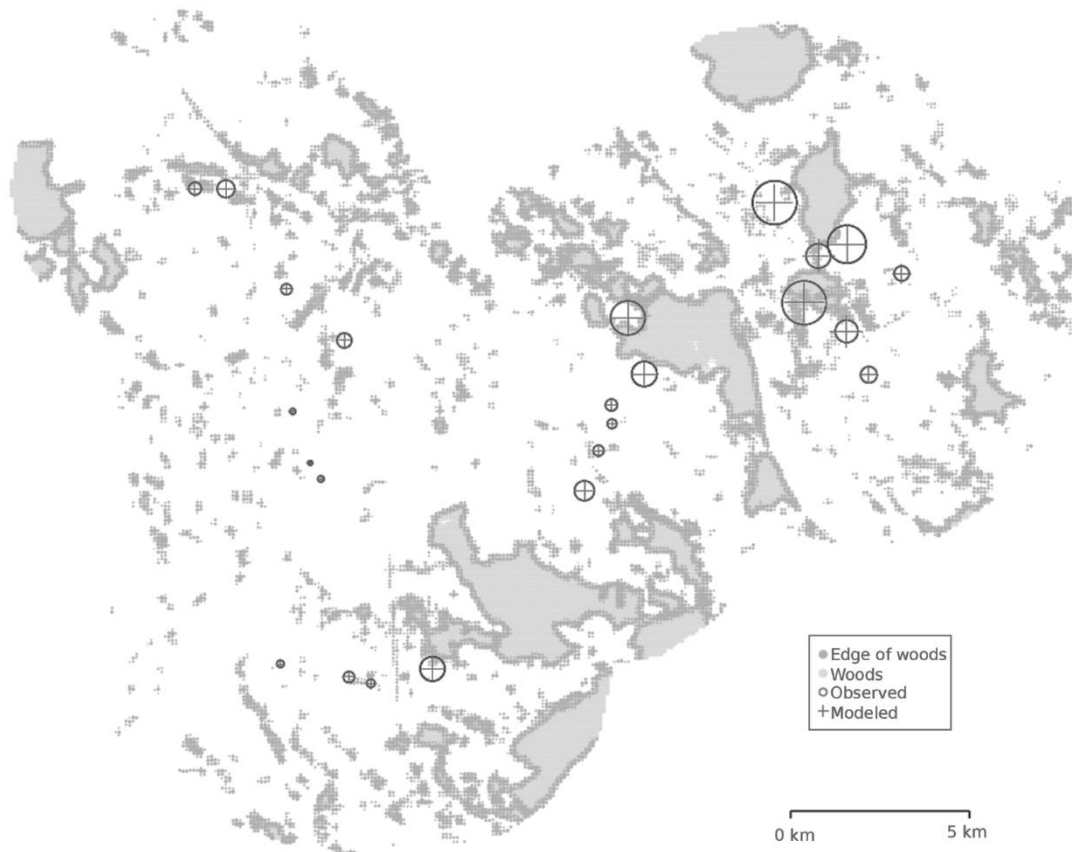


Figure 3. 3 : Abundance of pollen beetles observed (circle) and calculated (cross) by the model structured by site and in the context of the surrounding woodlands. Cross and circle sizes were proportional to pollen beetle abundance per 10 plants x 5 points x 4 observations. The surface of the wood area points (edge and core) was proportional to the area of woods in the pixel, with the maximum diameter equal to the pixel width and corresponding to 1ha.

To evaluate if the observed abundance spatial patterns we based our estimations of dispersal on were representative of what can be observed in previous studies [12], [14], [31] we also performed standard buffer based regressions. Specifically we used a generalized linear model (glm) describing the abundance as a simple function of the area of woodlands in buffers of increasing sizes. The best glm model was the model with a buffer radius of 1.8 km.

## 4. Discussion

Many studies have already demonstrated the importance of woodlands in pollen beetle life cycle [14], [15], studied the spatio-temporal patterns of pollen beetle distribution in crops [21] or modeled the immigration of pollen beetle into crops [32] here we explicitly modeled the immigration from woodlands into OSR fields estimating the average dispersal distance and testing additional hypothesis on this dispersal. We estimate the woodlands to be a source of pollen beetle proportionally to their size though the contribution of the center of large woodlands remains unclear. With the weeks, the gradient of pollen beetles from woodlands tended to fade out which translate in higher dispersal distances estimated. The pollen beetle likely dispersed with a similar mean dispersal distance in simple or complex landscapes. However, in complex landscapes, the estimation of the dispersal seems to have been obfuscated by other non-measured factors. The variability of the abundance between fields was largely explained by the dispersal from neighboring woods but there was as considerable remaining variability between dates, sites and, to a lesser extent, between fields.

Dispersal from the woodlands continued in OSR fields, but within the fields we observed a high variability of the abundance that is not explained by the distance to surrounding woodlands. Models accounting or not for the exact position of the sampling point in the field had similar results suggesting the gradient within fields was neither flat nor as strong as between fields. The high variability of the abundance within fields suggests that within fields the abundance is strongly influenced by other factors here not assessed. This would be nevertheless surprising given that the aggregation of pollen beetles in fields has been documented [33]. The OSR plants might have different attractiveness as pollen beetles abundance is correlated with the OSR growth stage [24]. Pollen beetles also tend to aggregate on blossomed OSR flowers, and flowering is not homogenous in the field [13]. Other studies found more pollen beetles at the field edges than at the center [20], [32] (six times more pollen beetles at the field edges). We might not see such a strong effect because OSR fields in our study were smaller: mean area of 25 ha, compared to 45 ha for [20], leaving less distance for the gradient to express itself. Alternatively, as other studies suggest that pollen beetle fly upwind into crops [16], transects might observe gradients of varying strength depending on the existence of a strongly prevailing wind and on the alignment of the transect to such prevailing wind.

Our estimation of the effect of the distance in the different sites suggests that the dispersal is similar in complex and simple landscapes, nevertheless the effect of the woodlands on the abundance can be obfuscated in complex landscapes, but only at early dates. Difficulties to

observe a statistical relationship between woodland areas and pollen beetle though unusual is not unprecedented [16]. In our particular case this effect is only hidden in complex landscapes and at early dates, a possible explanation could be that complex sites present more small overwintering sites such as hedgerows or grasslands [11]. The emergence of the pollen beetle happens when a sum of degree per day is attained [2], [16]. As non-wooded areas and even small wooded areas such as hedgerows warm up faster [34], pollen beetles overwintering into them might emerge faster and hence be early colonizer of OSR crops. In our complex landscape sites that had more grassland and hedgerows in the landscape, these potentially early colonizer could quickly spread all over the site blurring the nascent gradient of abundance from the woodlands. One could think that the semi-natural habitats such as hedgerows also had plentiful flowers and affected the dispersal as they might serve as relay providing resources to pollen beetle leaving their overwintering habitat, nevertheless to blur the signal only for the first dates of observations as we observed, such relay would have to 1) increase the dispersal a lot and 2) increase it only for the first pollen beetle emerging, neither proposition being sustained by published data to our knowledge, we hence favor our first hypothesis.

We find an important time effect with a maximum abundance of pollen beetle at the third date. An overall bell shape is expected as the wave of pollen beetle getting out of overwintering habitats swashes the neighboring habitats [12], [16] in addition crop attractiveness is maximal at blossom favoring such maximum of abundance [24]. Finally we observe very few pollen beetle on the second date that corresponds to a rainy and windy day limiting the observability of the pollen beetle [16].

As pollen beetles oviposit in buds, the growth stage of the OSR is a major parameter for the damage pollen beetles could cause. As the growth stage is linked to the date, early arriving pollen beetles are more likely to find non blossomed flowers and damage it [6], [13]. We found, at least in the simple landscapes, a significant effect of time on the dispersal range, confirming our hypothesis that with time they tend to move further away from woodlands. Alternatively, the dispersal might be very quick but highly density dependent: as the later dates are the one with more pollen beetles, they might go further when they are more numerous to emerge. Finally, the flight dispersal could also be temperature dependent; with warmer temperatures towards the end of the season pollen beetles are more likely to fly and could fly further [35]. . In any case, the fields closer to the woods are then not only attacked by more pollen beetles but also when the crops are most sensitive to the attacks [36]. This could extend to OSR fields close

to hedgerows or even grasslands if as suggested above hedgerows and grasslands are a significant source of pollen beetle early in the spring.

Overall, our study suggests a mean dispersal distance of 1.2 km, confirming a posteriori that the mapping of the woodlands within five kilometers of the OSR was enough. With both the Gaussian and the exponential kernels, we found a mean dispersal distance of 1.2 km, even if the exponential kernel had a fatter tail. In any case, these results suggest that dispersal movements are local [6] and that high abundance should rarely be found at ten kilometers from over-wintering sites. This estimation is largely in agreement with some estimations of pollen beetles dispersal based on mark-release-recaptures experiments [18] but differs from other estimates suggesting a dispersal distance of ten kilometers in two days [17]. One can note the very artificial conditions of release of these pollen beetles in mark-release-recapture. The feeding status and stress level of the insects as well as the date of release might have a strong impact on the dispersal; at the latest date, the average dispersal of 2.6 km we estimate in simple landscapes would be for example much more compatible with occasional flight over 10 km. Other statistical studies associated the abundance of pollen beetles with the area of woodlands within 1.5 to 2 kilometers of the sampling point [12], [14], [31]. This is in complete agreement with the maximum of likelihood we find in our data for models based on buffers of 1.8 km radius despite the fact that we observed much lower pollen beetle abundance per plant, suggesting that our estimate of dispersal is relevant for other times and places.

We found that the Gaussian kernel is slightly better than the exponential kernel and the conclusions are similar using one or the other kernel. The Gaussian kernel corresponds to a random dispersal while the exponential kernel corresponds to a centrifuge, dispersal from woodlands, with random stopping time [26]. The dispersal of the pollen beetles might be closer to the former. This is in agreement with [17], suggesting that *B. aeneus* fly in all the directions, with no effect of wind or topography. Nevertheless, upwind anemotaxis toward oilseed rape has been reported for overwintered *B. aeneus* [2], [16], [37]. In regions with a strong prevailing wind, using such symmetric kernels might not be adequate and more generally estimating a mean dispersal might not be relevant without considering the direction of the wind. Nevertheless the pollen beetle tend to not fly in strong winds, limiting the impact of strong dominant winds and in our case, in eastern Normandy, there is no clearly prevailing wind [38]. The use of pesticides could also potentially have a strong impact on the abundance and dispersal of the pollen beetle. In particular, a generalized use of insecticide on OSR before blossom followed by a diminution of the insecticide use after blossom could induce both the increase of

the observed population of pollen beetle with time and perturbation of the dispersal gradient early on. One could also argue that the OSR is more treated where pollen beetles are more abundant. This would tend to erase the gradient we observe from woodlands leading to higher estimated dispersal distance. It could be an explanation of the limited gradient we observed in complex landscapes. Nevertheless, overall, we observe a very clear gradient and the dispersal distance we estimate tend to be small compared to previous estimations by mark-release-recaptures. In any cases, our estimates are relevant for landscapes with similar insecticide pressure and conclusions on the dispersal of pollen beetle in landscapes fully exempt from insecticides might be significantly different. Abundance and potentially dispersal might also be affected by other OSR management practices such as cultivar used [8] or sowing dates as it impacts the flowering dates [39]. We do not expect those to be spatially correlated to the presence of woodlands limiting the impact on our estimates of the dispersal distance from woodlands to OSR. In general the overall stability of the dispersal distance estimates over the different sites at least for the later dates suggests a limited impact of the disparity of these practices in the landscape on the dispersal distance.

Depending on the model the source of pollen beetle to consider to best explain the data is the 100 m wide circumference of the woodlands or the whole woodlands. This surprising inversion might be due to a confusion between the site effect and the presence of woodlands large enough to have a core of a significant size within the 100 m circumference (Fig 3). It might also be linked to a higher semi-natural habitat density in the complex landscapes. In any case, there is no strong support for having less pollen beetles in the center of the woodlands. This is coherent with previous work finding that the pollen beetles overwinter at similar densities in edge under 10 m and at 50 to 100 m into woodlands [11]. The variability of *B. aeneus* capture related to the date (strong diminution of pollen beetles abundance the first of April) also confirms the impact of the weather on the sampling which was realized under light rain. This also confirms that rains can limit pollen beetles damage on OSR [17], [40].

We focused here on the dispersal distance of the pollen beetle from woodlands to OSR fields. Understanding how other land use might influence the dispersal could be key to manage the pollen beetle invasions. In particular, though the kernel based formalism we use does not imply that the dispersal is direct from the woodlands to the OSR fields, it might be beneficial to account explicitly for putative relay such as grasslands, hedgerows or wild flowers on the road borders [2] Such refinements of our model could help explain the differences observed between the sampled sites, currently aggregated in our catch all categorical term  $\gamma_g$ . It would imply to

better cartography the land uses but would also very significantly increase the computation time for the simulations. The abundance of pollen beetles overwintering in woodland could also vary according to the presence of previous OSR fields close to overwintering sites [16]. The surface of the OSR of the year N-1 at proximity of the woodlands could influence the load in these woodlands and participate to the site effect. Furthermore, woodlands and hedgerows characteristics such as moisture, exposition or litter thickness are important for pollen beetle overwintering [11].

Finally, natural enemies such as predators [41], [42], fungus [43], or parasitoids [42] may limit the growth of pollen beetle population. It could be argued that the observed patterns of abundance might be shaped by the predation of the pollen beetles by generalist predators such as birds. This is unlikely though as predation, as well as parasitism is expected to appear mostly around semi-natural landscapes and woods and hedgerows in particular [12], in clear opposition with the higher abundance observed here close to woods and in complex landscape sites. A possible control by natural enemies more intense around woodlands does not seem to counter the strong direct impact of these features on the life cycle of the pollen beetle.

We show that the average dispersal of the pollen beetle is over 1.2 km, inducing a very strong dependence of their abundance to the abundance of nearby woodlands or potentially other overwintering sites [11]. Within field variability in pollen beetle abundance was high. Finding the right attractors allowing to use trap crops as a within field management strategy [7], [9], possibly with earlier flowering [44] or adequate volatile compounds [8] might be efficient though difficult. Complex landscape do not seem to have a clear impact on the dispersal of the pollen beetle from the woodland. As this dispersal is not stopped either by intermediary OSR fields using “trap fields” nearby woodlands to limit pollen beetles dispersal does not seem relevant. Nevertheless, OSR fields close to woodlands are both more infested and to some extent infested earlier than OSR fields far from woodlands. To reduce the risk of OSR field to be colonized by pollen beetle when they are close to woodlands, plantation of early OSR flowering varieties may be recommended.

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### Supplementary materials

Table S3.1 Summary of all the bayesian models

N°	Factor type	Origin	Same field point structure	Kernel	Site dependent dispersal	AIC	Mean $\beta_{dt}$	Mean Dmean (km)
1	Date+field	area	split	Gaus.	TRUE	2419.8	0.22 [0.07,0.38]	3.44 [1.59,5]
2	Date+field	area	split	exp.	TRUE	2424.0	0.58 [0.18,1.1]	4.18 [3.11,6.44]
3	Date+field	edge	split	Gaus.	TRUE	2427.7	0.2 [0.05,0.38]	3.06 [2.43,4.11]
4	Date+field	edge	split	exp.	TRUE	2432.1	0.52 [0.12,1]	3.59 [2.72,5.19]
5	Date:field	NA	none	Gaus.	FALSE	2433.7	NA	NA
6	Date+field	area	merged	exp.	TRUE	2435.4	0.52 [-0.05,1.4]	4.09 [2.98,6.57]
7	Date:field	area	split	Gaus.	TRUE	2435.6	0.37 [0.08,0.61]	1.59 [1.14,2.23]
8	Date:field	area	split	exp.	TRUE	2435.9	1 [0.25,1.67]	1.59 [1.07,2.65]
9	Date:field	edge	split	exp.	TRUE	2436.6	0.96 [0.24,1.73]	1.33 [0.87,2.02]
10	Date:field	edge	split	Gaus.	TRUE	2437.0	0.38 [0.04,0.64]	1.38 [0.91,1.97]
11	Date+field	edge	merged	Gaus.	TRUE	2439.5	0.12 [-0.09,0.4]	2.79 [1.28,3.8]
12	Date:field	area	merged	Gaus.	FALSE	2440.1	0.03 [-0.08,0.16]	2.23 [1.53,3.03]
13	Date:field	area	merged	exp.	FALSE	2440.3	0.09 [-0.25,0.52]	2 [1.32,2.97]
14	Date:field	area	split	Gaus.	FALSE	2440.4	-0.01 [-0.1,0.11]	2.44 [1.7,3.29]
15	Date:field	edge	merged	exp.	FALSE	2441.1	0.09 [-0.36,0.59]	1.73 [1.07,2.65]
16	Date:field	edge	merged	Gaus.	FALSE	2441.3	0.04 [-0.12,0.28]	1.87 [1.13,2.74]
17	Date:field	edge	split	Gaus.	FALSE	2441.8	0.01 [-0.14,0.21]	2.02 [1.28,2.87]
18	Date:field	area	split	exp.	FALSE	2441.9	-0.01 [-0.29,0.35]	2.23 [1.5,3.25]
19	Date:field	edge	split	exp.	FALSE	2441.9	0.08 [-0.34,0.94]	1.76 [1.02,2.73]
20	Date+field	edge	merged	exp.	TRUE	2442.3	0.49 [-0.15,1.35]	3.59 [2.65,5.4]
21	Date+field	area	merged	Gaus.	TRUE	2448.0	0.17 [-0.11,0.42]	1.45 [0.69,2]
22	Date:site	edge	split	Gaus.	TRUE	2453.3	0.26 [0.1,0.42]	1.24 [0.98,2.61]
23	Date:site	edge	merged	Gaus.	TRUE	2454.6	0.16 [0.04,0.29]	1.19 [0.98,1.69]
24	Date:site	edge	merged	Gaus.	TRUE	2454.9	0.17 [0.04,0.33]	1.2 [0.98,1.93]
25	Date:site	edge	merged	Gaus.	FALSE	2454.9	0.07 [-0.03,0.18]	1.09 [0.96,1.26]
26	Date:site	edge	merged	Gaus.	TRUE	2455.1	0.17 [0.04,0.31]	1.19 [0.98,1.7]
27	Date:field	edge	merged	exp.	TRUE	2457.2	0.18 [-1.51,1.73]	1.21 [0.71,2]

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28	Date:site	edge	split	exp.	TRUE	2457.8	0.61 [0.18,1.06]	1.31 [1,3.11]
29	Date:site	edge	split	exp.	TRUE	2458.0	0.61 [0.17,1.04]	1.35 [1.01,5.63]
30	Date:field	area	merged	exp.	TRUE	2458.3	0.15 [-1.2,1.43]	1.46 [0.91,2.47]
31	Date:site	edge	split	Gaus.	FALSE	2459.8	0.06 [-0.04,0.17]	1.11 [0.97,1.28]
32	Date:site	edge	merged	exp.	FALSE	2460.1	0.12 [-0.11,0.38]	1.16 [0.98,1.36]
33	Date:field	edge	merged	Gaus.	TRUE	2461.0	0.06 [-0.66,0.83]	1.21 [0.71,2.01]
34	Date:field	edge	merged	Gaus.	TRUE	2461.1	0.06 [-0.58,0.76]	1.22 [0.72,1.99]
35	Date:site	edge	split	exp.	FALSE	2462.4	0.13 [-0.12,0.44]	1.16 [0.99,1.37]
36	Date:site	edge	merged	exp.	TRUE	2462.9	0.37 [0.02,0.79]	1.27 [1,2.2]
37	Date:site	area	split	exp.	TRUE	2466.2	0.54 [0.14,0.95]	1.81 [1.3,3.04]
38	Date:field	area	merged	Gaus.	TRUE	2468.4	0.08 [-0.48,0.75]	1.47 [0.92,2.29]
39	Date:site	area	merged	exp.	FALSE	2469.1	0.1 [-0.1,0.32]	1.45 [1.23,1.74]
40	Date:site	area	merged	exp.	TRUE	2470.4	0.35 [0.04,0.72]	1.73 [1.27,4.75]
41	Date:site	area	split	Gaus.	TRUE	2471.0	0.23 [0.08,0.4]	1.77 [1.34,3.7]
42	Date:site	area	merged	Gaus.	TRUE	2472.4	0.15 [0.02,0.31]	1.77 [1.33,5.63]
43	Date+site	edge	merged	Gaus.	TRUE	2474.0	0.03 [-0.08,0.21]	2.35 [1.48,2.83]
44	Date:site	area	split	exp.	FALSE	2474.1	0.08 [-0.12,0.29]	1.48 [1.25,1.77]
45	Date:site	area	merged	Gaus.	FALSE	2475.7	0.04 [-0.04,0.13]	1.44 [1.26,1.66]
46	Date+field	edge	merged	exp.	NA	2478.4	0.47 [0.13,0.83]	1.65 [0.86,4.61]
47	Date+field	edge	merged	Gaus.	NA	2478.7	0.13 [0.02,0.25]	1.41 [0.85,2.8]
48	Date+field	edge	merged	exp.	NA	2479.2	0.3 [0.05,0.6]	1.46 [0.81,4.12]
49	Date+field	edge	merged	Gaus.	NA	2479.5	0.12 [0.03,0.24]	1.44 [0.88,2.95]
50	Date+field	edge	split	exp.	NA	2480.4	0.29 [0.05,0.61]	1.51 [0.8,3.5]
51	Date+field	edge	split	exp.	NA	2480.6	0.33 [0.06,0.69]	1.64 [0.8,3.52]
52	Date+field	area	merged	Gaus.	NA	2480.8	0.1 [0.01,0.21]	1.15 [0.69,1.84]
53	Date+field	NA	none	NA	NA	2480.9	NA	NA
54	Date+field	edge	split	Gaus.	NA	2481.4	0.12 [-0.01,0.26]	1.41 [0.79,4.09]
55	Date:site	area	split	Gaus.	FALSE	2481.5	0.02 [-0.05,0.1]	1.49 [1.29,1.71]
56	Date+field	edge	split	Gaus.	NA	2481.7	0.12 [0.01,0.25]	1.46 [0.82,2.97]
57	Date+field	edge	merged	exp.	NA	2487.4	0.07 [0.02,0.22]	-3.56 [-4.79,-2.58]
58	Date+field	edge	split	Gaus.	FALSE	2488.6	0.15 [0.04,0.27]	1.25 [0.78,2.13]
59	Date+field	edge	split	exp.	FALSE	2488.6	0.4 [0.12,0.75]	1.31 [0.74,2.71]
60	Date+field	area	merged	exp.	FALSE	2489.2	0.33 [0.05,0.65]	1.1 [0.71,1.82]
61	Date+site	area	split	exp.	TRUE	2489.7	0.58 [0.15,1.09]	2.38 [1.87,3.32]
62	Date+field	area	split	exp.	FALSE	2492.3	0.37 [0.08,0.74]	1.1 [0.71,1.87]
63	Date+site	area	split	Gaus.	TRUE	2493.1	0.26 [0.08,0.41]	2.12 [1.76,2.6]
64	Date+field	NA	none	Gaus.	FALSE	2493.7	NA	NA
65	Date+field	area	split	Gaus.	FALSE	2494.4	0.11 [0.02,0.27]	1.12 [0.72,1.71]
66	Date+site	edge	merged	Gaus.	NA	2495.2	0.15 [0.04,0.25]	1.07 [0.94,1.24]
67	Date+site	edge	split	Gaus.	NA	2498.0	0.15 [0.05,0.26]	1.07 [0.93,1.24]
68	Date+site	area	merged	exp.	TRUE	2498.9	0.3 [-0.05,0.79]	2.41 [1.93,3.32]
69	Date+site	area	merged	Gaus.	TRUE	2499.5	0.13 [0.03,0.3]	2.13 [1.79,2.64]
70	Date+site	edge	split	exp.	NA	2500.4	0.38 [0.11,0.67]	1.1 [0.94,1.33]
71	Date+site	edge	merged	exp.	NA	2501.2	0.35 [0.07,0.61]	1.12 [0.96,1.34]
72	Date+site	edge	split	exp.	TRUE	2502.1	0.27 [-0.2,1.01]	3.49 [1.66,4.83]
73	Date+site	edge	split	Gaus.	TRUE	2505.2	0.15 [-0.05,0.46]	2.91 [1.43,3.65]
74	Date+site	area	merged	exp.	NA	2507.2	0.32 [0.12,0.58]	1.37 [1.17,1.64]
75	Date+site	edge	merged	exp.	TRUE	2507.6	0.02 [-0.32,0.43]	3.54 [2.56,4.76]

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76	Date+site	edge	merged	Gaus.	TRUE	2510.3	0.03 [-0.12,0.26]	2.98 [1.52,3.59]
77	Date+site	area	merged	Gaus.	NA	2512.4	0.11 [0.03,0.21]	1.37 [1.19,1.58]
78	Date+site	area	split	Gaus.	NA	2517.4	0.11 [0.02,0.2]	1.38 [1.21,1.59]
79	Date+site	area	split	exp.	FALSE	2523.3	0.24 [0.03,0.49]	1.4 [1.19,1.68]
80	Date	edge	merged	Gaus.	NA	2568.2	0.09 [-0.02,0.18]	1.09 [0.95,1.25]
81	Date	edge	split	Gaus.	NA	2571.9	0.08 [-0.03,0.2]	1.09 [0.95,1.27]
82	Date	edge	merged	exp.	NA	2574.8	0.2 [-0.07,0.49]	1.12 [0.96,1.33]
83	Date	edge	split	exp.	NA	2576.1	0.2 [-0.06,0.5]	1.12 [0.96,1.34]
84	Date	edge	merged	exp.	NA	2576.1	0.2 [-0.06,0.5]	1.12 [0.96,1.34]
85	Date:site	edge	merged	Gaus.	TRUE	2589.1	0.1 [-0.34,0.52]	0.5 [0.34,1.27]
86	Date	edge	split	exp.	NA	2593.1	0.35 [-0.04,0.76]	0.95 [0.79,1.17]
87	Date:site	NA	none	Gaus.	FALSE	2643.9	NA	NA
88	Date:site	NA	none	Gaus.	FALSE	2643.9	NA	NA
89	Date+site	NA	none	NA	NA	2685.2	NA	NA
90		1 area	split	Gaus.	TRUE	2735.3	0.24 [0.11,0.53]	5 [2.13,6.6]
91		1 area	split	exp.	TRUE	2737.4	1.48 [0.68,2.29]	2.63 [1.35,5.62]
92		1 area	merged	exp.	TRUE	2737.9	0.92 [0.55,2.42]	4.91 [2.15,6.32]
93		1 area	merged	Gaus.	TRUE	2751.9	0.29 [0.16,0.53]	4.12 [2.07,4.89]
94		1 edge	split	exp.	TRUE	2752.9	0.75 [0.5,1.73]	6.46 [3.9,7.89]
95	Date+site	NA	none	Gaus.	FALSE	2755.2	NA	NA
96		1 edge	split	Gaus.	TRUE	2765.0	0.26 [0.17,0.43]	5.03 [3.09,6.04]
97		1 edge	merged	Gaus.	TRUE	2767.0	0.25 [0.17,0.4]	5.1 [3.47,6.16]
98		1 edge	merged	exp.	TRUE	2768.9	2.85 [1.44,3.74]	4.12 [3.18,5.2]
99	field	edge	split	Gaus.	TRUE	2769.5	0.18 [0.03,0.43]	4.19 [2.5,6.23]
100		1 area	merged	exp.	FALSE	2789.3	1.09 [0.74,1.45]	1.45 [1.15,1.81]
101		1 area	split	exp.	FALSE	2791.7	1.09 [0.73,1.48]	1.45 [1.14,1.8]
102	Date	NA	none	Gaus.	FALSE	2799.0	NA	NA
103	Date	NA	none	Gaus.	FALSE	2799.0	NA	NA
104		1 area	merged	Gaus.	FALSE	2799.8	0.34 [0.22,0.47]	1.37 [1.13,1.69]
105		1 area	split	Gaus.	FALSE	2802.1	0.36 [0.22,0.52]	1.39 [1.14,1.71]
106	field	area	split	Gaus.	TRUE	2808.8	0.42 [0.26,0.64]	2.59 [1.41,4.11]
107	field	area	merged	exp.	TRUE	2822.2	2.76 [1.67,3.75]	0.26 [0.18,0.78]
108	field	NA	none	Gaus.	FALSE	2823.4	NA	NA
109	field	NA	none	Gaus.	FALSE	2823.4	NA	NA
110	field	area	merged	Gaus.	TRUE	2826.4	0.72 [0.44,1.07]	0.42 [0.24,1.01]
111	field	area	merged	Gaus.	FALSE	2826.7	0.69 [0.52,0.84]	0.33 [0.27,0.42]
112	field	edge	merged	exp.	TRUE	2828.7	2.96 [1.94,3.93]	0.23 [0.17,0.43]
113	field	area	merged	exp.	FALSE	2832.3	2.06 [1.47,2.68]	0.34 [0.25,0.51]
114	field	edge	merged	Gaus.	TRUE	2834.7	0.77 [0.47,1.1]	0.26 [0.21,0.63]
115	field	edge	split	exp.	TRUE	2835.6	1.37 [0.75,2.21]	2.98 [1.99,5.65]
116		1 edge	merged	exp.	FALSE	2837.9	1.13 [0.73,1.57]	1.2 [0.94,1.55]
117	field	edge	merged	Gaus.	FALSE	2838.3	0.69 [0.5,0.86]	0.29 [0.25,0.36]
118		1 edge	split	exp.	FALSE	2839.9	1.11 [0.65,1.59]	1.19 [0.94,1.54]
119		1 edge	merged	Gaus.	FALSE	2841.4	0.33 [0.19,0.47]	1.14 [0.95,1.42]
120		1 edge	split	Gaus.	FALSE	2842.6	0.34 [0.2,0.51]	1.14 [0.95,1.43]
121	field	edge	merged	exp.	FALSE	2842.7	2.33 [1.68,2.95]	0.3 [0.23,0.42]
122	field	area	split	exp.	TRUE	2844.8	1.66 [1.08,2.43]	0.92 [0.53,1.47]
123	field	area	split	exp.	FALSE	2845.9	1.68 [1.23,2.19]	0.49 [0.38,0.68]

### Chapitre 3 : Caractérisation de la dispersion des méligèthes

124	field	area	split	Gaus.	FALSE	2852.3	0.59 [0.42,0.75]	0.49 [0.4,0.63]
125	field	edge	split	exp.	FALSE	2858.6	1.85 [1.33,2.41]	0.44 [0.35,0.58]
126	field	edge	split	Gaus.	FALSE	2865.2	0.62 [0.43,0.8]	0.44 [0.37,0.55]
127	site	NA	none	Gaus.	FALSE	2934.9	NA	NA
128	site	NA	none	Gaus.	FALSE	2934.9	NA	NA
129	Date	area	split	Gaus.	TRUE	2959.8	0 [-0.11,0.1]	1.69 [1.26,2.54]
130	Date	area	merged	Gaus.	TRUE	2960.5	-0.02 [-0.13,0.09]	1.67 [1.23,2.5]
131	Date	area	split	exp.	TRUE	2978.0	-0.02 [-0.32,0.27]	1.65 [1.17,2.57]
132	Date	area	merged	exp.	TRUE	2980.3	-0.1 [-0.44,0.18]	1.67 [1.16,2.58]
133	Date	area	merged	Gaus.	FALSE	2988.6	0.02 [-0.06,0.09]	1.36 [1.19,1.59]
134	Date	area	merged	exp.	FALSE	2993.0	-0.03 [-0.22,0.14]	1.42 [1.21,1.69]
135	1	NA	none	Gaus.	FALSE	2996.8	NA	NA
136	1	NA	none	Gaus.	FALSE	2996.8	NA	NA
137	Date	area	split	Gaus.	FALSE	2998.4	0 [-0.09,0.08]	1.4 [1.23,1.63]
138	Date	area	split	exp.	FALSE	2998.9	-0.03 [-0.22,0.16]	1.43 [1.22,1.71]
139	Date	edge	split	Gaus.	TRUE	3111.9	0.24 [-0.07,0.43]	1.67 [1.05,2.28]
140	Date	edge	merged	Gaus.	TRUE	3113.9	-0.02 [-0.2,0.09]	1.66 [1.07,2.02]
141	Date	edge	split	exp.	TRUE	3129.8	0.52 [-0.3,1.08]	1.71 [0.93,2.6]
142	Date	edge	merged	exp.	TRUE	3138.7	-0.15 [-0.76,0.24]	1.67 [0.95,2.56]
143	site	area	split	Gaus.	TRUE	3388.0	0.16 [-0.01,0.35]	2.63 [1.81,4.65]
144	site	area	merged	Gaus.	TRUE	3392.2	0.22 [0.02,0.45]	2.04 [1.08,3.56]
145	site	area	split	exp.	TRUE	3400.9	0.47 [-0.01,1.07]	2.96 [1.99,5.99]
146	site	area	split	exp.	TRUE	3400.9	0.47 [0.01,1.09]	2.97 [1.99,6.4]
147	site	area	split	Gaus.	FALSE	3406.2	0.35 [0.22,0.47]	1.23 [0.99,1.45]
148	site	area	merged	Gaus.	FALSE	3409.6	0.31 [0.19,0.42]	1.25 [1.01,1.48]
149	site	edge	split	Gaus.	TRUE	3410.5	0.14 [-0.03,0.35]	2.29 [1.53,4.42]
150	site	area	merged	exp.	TRUE	3410.5	0.43 [-0.01,1.09]	2.85 [1.99,5.94]
151	site	area	split	exp.	FALSE	3413.7	0.7 [0.43,0.99]	1.2 [0.99,1.44]
152	site	edge	merged	Gaus.	TRUE	3414.9	0.11 [-0.06,0.33]	2.43 [1.6,4.67]
153	site	area	merged	exp.	FALSE	3423.2	0.57 [0.32,0.83]	1.26 [1.05,1.5]
154	site	edge	split	Gaus.	FALSE	3426.4	0.28 [0.14,0.42]	0.94 [0.8,1.13]
155	site	edge	split	exp.	TRUE	3426.8	0.39 [-0.1,1.03]	2.63 [1.72,4.96]
156	site	edge	merged	Gaus.	FALSE	3429.8	0.23 [0.1,0.36]	0.96 [0.83,1.15]
157	site	edge	merged	exp.	TRUE	3437.3	0.36 [-0.16,1.08]	2.66 [1.74,5.64]
158	site	edge	split	exp.	FALSE	3439.7	0.65 [0.32,0.99]	1.01 [0.85,1.21]
159	site	edge	merged	exp.	FALSE	3450.8	0.51 [0.22,0.78]	1.08 [0.91,1.29]



## Chapitre 4

Où se trouvent les méligèthes lorsqu'ils  
ne sont pas dans les parcelles de colza et  
dans les bois ?





## **Chapitre 4 : Où se trouvent les méligèthes lorsqu'ils ne sont pas dans les parcelles de colza et dans les bois ?**

Dans le chapitre précédent, nous avons montré que les méligèthes parcourent en moyenne 1,2 km en sortant des bois, des estimations cohérentes avec les résultats trouvés à partir de données génétiques dans les chapitres précédents. Une zone d'ombre persiste sur où se trouvent les méligèthes lorsqu'ils ne sont ni dans les bois, ni dans les parcelles de colza. L'objectif de cette partie était de pallier à ce manque d'information sur leur répartition dans le paysage et sur les déterminants de cette répartition.

Les zones non cultivées sont des éléments clés pour de nombreuses espèces importantes pour la santé des cultures, qu'il s'agisse de ravageurs ou d'ennemis naturels. Ils peuvent être des abris mais aussi des sources de nourriture comme le pollen. C'est aussi le cas pour les fleurs en bordure des champs ou des adventices dans les champs. Ces ressources en pollen ont été étudiées pour évaluer leur rôle dans la dynamique des populations de méligèthes. Pour quantifier la présence et l'attraction des méligèthes dans d'autres habitats que les bois ou les parcelles de colza, nous avons dressé la liste et estimé le pourcentage de recouvrement des espèces florales sur 32 bords de route, 14 prairies, 6 jachères et 17 parcelles en culture à l'aide de la méthode des quadrats et échantillonné des méligèthes dans chacun de ces habitats.

L'article suivant qui présente ce travail sera prochainement soumis à une revue à comité de lecture en écologie et s'intitulera : « **Wild flowers, important pollen source for an oilseed rape pest** ».



## **Wild flowers, important pollen source for an oilseed rape pest**

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### **Abstract**

The non-crops areas are key elements for many species of importance for crop health, whether they are pests or natural enemies. They can be shelters but also sources of food such as pollen. It is also the case for flowers at the edges of fields or weeds. Understanding the role of such resources for *Brassicogethes aeneus* (Fabricius) (Coleoptera), a major pest of the oilseed rape, during its dispersal in rural landscapes might help to design innovative pest control. This species has two phases of dispersal during which it is likely to feed on pollen. In early spring it leaves its overwintering sites (woodlands) to breed in the oilseed rape buds. Early in the summer, the new generation emerges from oilseed rape fields and then seeks overwintering sites. Here, to assess where pollen beetle stop during those migrations and what attracts them, we sampled pollen beetles, list and estimated percentage of cover of flower species in 32 roadsides, 14 grasslands, 6 fallows and 17 fields using the quadrats method. We show that in the spring, the few pollen beetles emerging before the flowering of oilseed rape are present in all the habitats but are then almost only found into oilseed rape fields. In the summer they were principally found on the weeds of crop fields and some extent in grasslands and fallows. This summer flight is over in less than a month. In both seasons *B. aeneus* individuals were attracted by areas with flowers. In the spring, they were attracted by yellow flowers with easy access to pollen. In the summer though not all flowers had the same attractiveness we could not identify specific attractive traits. These results highlight the role of weeds in crop areas, and to a lesser extent of non-crop areas and the importance of an adapted management further and beyond the host crop.

Key words: *Brassicogethes aeneus*, grasslands, oilseed rape, roadsides, weeds, wild flowers

## 1. Introduction

The proximity of crops and non-crops areas such as, grassland, woodlands, hedgerows or roadsides, is common in agricultural landscapes. Because these habitats are less disturbed than annual crop fields, they provide a range of resources for natural enemies, including food, alternative prey or hosts, shelters and overwintering sites (Geiger et al., 2009; Bianchi et al., 2006; Landis et al., 2000). For example, some carabid beetles use grasslands as overwintering habitats (Purtauf et al., 2005). Spider abundance might also be enhanced by high percentages of non-crop habitats near the crops (Schmidt & Tschardt, 2005). However, some crop pests can also use them as feeding, sheltering or overwintering sites (Bianchi et al., 2006), though the behavior of insect pests outside of their host plant might usually be well known.

It is the case for pollen beetles, *Brassicogethes aeneus* (formerly named *Meligethes aeneus*), one of the major oilseed rape (OSR) pests in Europe (Alford 2003). In the spring, when the temperature reaches 12°C (Williams, 2010), overwintered pollen beetles emerge from the woodlands to then migrate into the OSR fields causing damages by both oviposition and feeding in buds (Hansen et al., 2004). Then the larva feed on the flowers, and when the development end, it drop to the ground to pupate. The new generation emerges in summer and seeks overwintering sites. Knowledge on where go pollen beetles and which characteristics of wild flowers attract them are weak. During the spring dispersal, some studies showed pollen beetles might eat pollen from dandelions (Taimr et al., 1967), and from other plant species that grow at the verges of fields (Free and Williams, 1978), urban areas and nature reserves (Ouvrard et al., 2016). In summer, some studies (Free and Williams, 1978, Ouvrard et al., 2016) showed that pollen beetles feed on a wide range of flowers from field margins. To the best of our knowledge the flowers from other surfaces such as grasslands, fallows or fields (weeds), have not been studied as resources for pollen beetles. The overwintered individuals of *B. aeneus* are known to be attracted by oilseed rape flowers, and particularly by their color, yellow (Jönsson et al., 2007) and their pollen (Cook et al., 2004) but such studies have not been extended to wild flowers. Understanding where pollen beetles go during their dispersal phases could help to manage them.

We first aimed at determining which habitats are a relay for pollen beetles and if landscape around these habitats influences pollen beetles numbers in these habitats. Secondly, we investigated which flowers are preferentially visited by *B. aeneus* in the non-crop habitats previously identified and if the characteristics of these flowers, identified for OSR also apply

to wild flowers. To this effect, we sampled 7 times in 2016 (three in the spring, four in the summer) pollen beetles and flowers in quadrats of crops and non-crop habitats such as the roadsides of woodlands and fields, fallows and grassland in an agricultural landscape of Northern France.

## **2. Material and methods**

### ***2.1. Study sites***

We conducted our study in an agricultural landscape with an extent of approximately 8 km x 6 km in the Normandy region, France (48°58'29.5"N 1°26'24.4"E) (Figure 4.1a) in the spring and summer 2016. The semi-natural habitats consist in large woodland area and small woodlots, hedgerows and grasslands. The dominant crops are wheat, barley and oilseed rape. We sampled grasslands, fallows, roadsides along woodlands and fields and other crop fields. Those areas were chosen to represent the diversity of the rural landscape the pollen beetles disperse in, excluding the center of the woodland areas, already known to be the origin of the spring dispersal and the destination of the summer dispersal. In the spring, on the 24<sup>th</sup> of March we selected 14 roadsides along fields, 11 roadsides along woodlands, 7 grasslands and 6 fallows. On April 6<sup>th</sup> we chose 5 other roadsides along fields and 3 roadsides along woodlands and 7 other grasslands. The 20<sup>th</sup> April we sampled all these areas (Figure 4.1b), Tableau 4.1). At the same dates, we sampled adult pollen beetle by beating ten randomly chosen plants inside plastic bags at 10 points in 5 OSR fields. In the summer, we sampled all these areas, except OSR fields that were dry and impenetrable, at five dates between the 8<sup>th</sup> June and 7<sup>th</sup> July and we added 17 edges of fields (wheat, barley, OSR, medicago, pea).

### ***2.2. Insect sampling***

We sampled pollen beetles in quadrats of 60 cm x 60 cm in diverse areas. We sampled 5 quadrats per area. In each of those quadrats, the pollen beetles were captured by beating flowers and we listed the flower species presenting pollen beetle. Among the 15,335 pollen beetles captured, 230 individuals equally distributed among the diverse sites were identified by observing their meta-femur (Audisio 1993). Most pollen beetle were identified as *B. aeneus* (99 %).

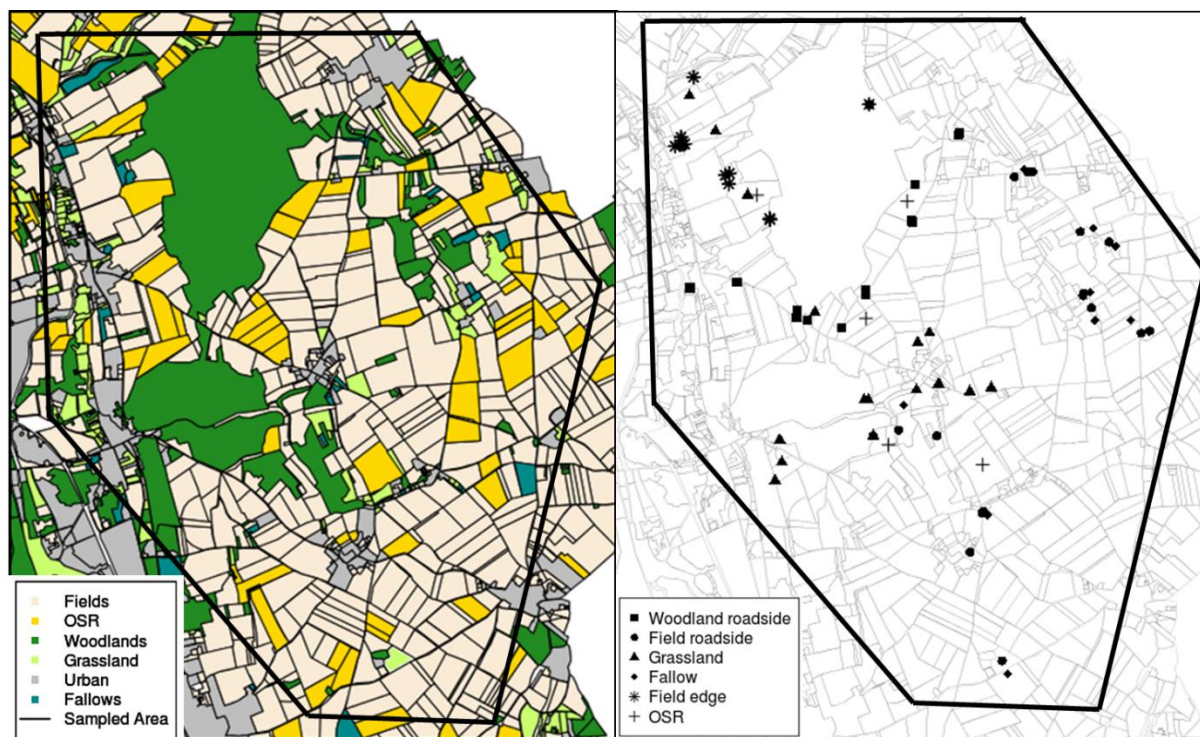


Figure 4.1 : Map of the studied site (Normandy region, France), describing a) land uses around the sampled points in 2016 and b) the location of the sampled points.

Tableau 4.1 : Habitat-sites sampled in spring and summer 2016. Sites of the 03/24/2016 were different of site of the 04/06/2016 and were merged for other sampling dates.

	Spring			Summer			
	03/24/1	04/06/201	04/20/1	06/06/201	06/15/1	06/22/1	07/07/201
	6	6	6	6	6	6	6
Grassland	7	7	14	14	14	14	14
Fallow	6	0	6	6	6	6	6
Roadside	25	8	23	23	23	23	23
OSR	5	5	5	0	0	0	0
Field edge	0	0	0	0	17	17	17

### 2.3.Measures of vegetation

In each quadrat, we measured the percentage of cover of each plant species. Based on the BiolFlor data base (Klotz et al., 2002), we categorized each flower species according to its amount of pollen in three classes, absence of pollen (none), presence of pollen (present), and large amount of pollen (plenty) (Table S1). We performed the same classification for the amount of nectar. Finally, we categorized flowers by their shape (Bell, lip, dis, ray, disc-ray, funnel, and flag) and color (Table S1).

#### *2.4. Mapping and geomatics*

We mapped forests and field delimitations within three kilometers from sampled fields using aerial photographs (GoogleMyMaps pixel size: 0.5 m, ©2017 TerraMetrics). We also mapped OSR fields within three kilometers of the selected sampling fields by visiting the area. We calculated woodland and OSR proportions around each sampling point within six buffer radius of 0.5 to 3 km using the R package rgeos (Bivand & Rundel 2013) using Universal Transverse Mercator (UTM) projection. In subsequent statistical analysis, we used the normalized log-transform of the proportions of OSR and woodlands in these buffers.

#### *2.5. Statistical analysis*

First we compared the attractiveness of habitats to pollen beetles, both in the spring and the summer we performed a generalized linear model (glm) of the abundance of pollen beetle by quadrat as a function of the habitat. We also accounted for the proportion of OSR and woodlands and the date. The habitat and the date were considered as random effects. We used a negative binomial regression as the count data were overdispersed. As there were many quadrats with no pollen beetles, frequentist regressions would not converge and we used Bayesian regressions with the rstanarm package (Stan Development Team 2016). We selected the buffer radius for the effect of OSR and woodlands by performing this model with each of the combinations possible of the buffer radius of OSR and woodland proportion and selected the best according to the widely applicable information criterion (WAIC) as implemented by the package loo (Vehtari et al., 2016).

Second, we explored the determinants of the attractiveness of the different habitats. We used the same model as previously but added either the total percentage of coverage by flowers in the quadrat, the number of flower species in the quadrat, the presence or absence of each flower species, the percentage of cover of each flower species. As the goal was to determine where were pollen beetles when they were not in OSR, we did not include pollen beetles caught in the OSR fields in these models.

Finally, to see if some characteristics of the flowers were more susceptible to attract pollen beetles in the quadrat we fitted the same model but using the percentage of cover of characteristics in place of flowers. The characteristics were the color, the shape and the presence or absence of nectar and pollen (Table S1). We also performed this model with the mere presence or absence of these characteristics in the quadrat. All these models were performed for both seasons and were performed three times with different starting values for the MCMC



chains (Geweke, 1991) and compared using the WAIC. For the prior of the regression coefficient, we used a centered normal distribution with standard deviation of 2.5 and for the prior of the intercept we used also a centered normal distribution with a standard deviation of 5.

In addition to the above analysis on the determinants of the presence of pollen beetle in the quadrat we characterized the repartition of the pollen beetle over the different flower species present in the quadrat. As we had noted the fact that pollen beetle were found or not on each flower species for each quadrat we modelled the probability of a flower species to present pollen beetles weighted by the log of the percentage of flowers in the quadrat. To estimate this probability, we estimated a factor of attraction of each flower species.

On each quadrat  $q$ , we consider each pollen beetle  $i$  to be on a flower species  $f$  with a probability  $p_{qf}$  proportional to the attraction factor of the flower species and inversely proportional to the sum of the attraction factors of the species present in the quadrat:

$$p_{qf} = \frac{F_{fq} \cdot w_f}{\sum_j F_{jq} \cdot w_j}$$

where  $F_{fq}$  is 1 if the flower species  $f$  is in the quadrat  $q$  and 0 if not and  $w_f$  is an attraction factor consistent over the quadrats. We also tested a very similar model accounting for the percentage of cover for each flower species in the quadrat. In this case  $F_{fq}$  becomes the cover for the flower species  $f$  in the quadrat  $q$ .

With  $n_q$  pollen beetle, considered to choose the flowers independently, the distribution of the pollen beetle over the flower species is multinomial. As we only have the presence or absence on each flower species, we describe it with a multinomial distribution of presence with  $n_q$  draws without replacement with the  $p_{qf}$  probabilities presented above. We implemented the computation of this probability in the `ppamultinom` function (package `MFSAS`, Barbu 2017a). We then estimate the attraction factors ( $w_{fs}$ ) with a bayesian MCMC. We used as prior for the  $w_{fs}$  a log-normal distribution centered on the log scale. We tested three standard deviations on the log scale: 1, 2 and 5. Each MCMC was run until the Geweke diagnostic (Geweke, 1991) and the Raftery and Lewis diagnostic (Raftery & Lewis, 1992) were satisfied as implemented in the `YAMH`, R package (Barbu, 2017b). All geomatics treatment and statistical analyses were performed using R software (R Core Team 2016).

### 3. Results

Overall, we captured 4159 pollen beetle in the spring (3351 not in OSR) and 7825 in the summer. The number of pollen beetles by quadrat varied from 0 to 160 (mean = 8.33) in the spring and from 0 to 450 (mean = 6.16) in the summer. We listed 58 species of flowers in the quadrat, respectively 13 and 25 of them presented pollen beetle in the spring and in the summer (table S4.1). Percentage of cover between habitats were highly variable among and between sites (Table S4.2).

In quadrat without flowers, no pollen beetles were found, and pollen beetles were found only on flowers in both seasons. We sampled 20 % and 63 % of the quadrats in the spring and in the summer respectively without flowers and pollen beetles. The densities (abundance of pollen beetle per quadrat) were highly variable during both seasons (Figure 4. 2).

#### *3.1. Where are pollen beetles in the spring?*

In the spring, densities reached a pic on April 6<sup>th</sup> (Figure 4. 2a). In the summer, there were no pollen beetles 6<sup>th</sup> June then the densities increased until June 22<sup>th</sup> and strongly decreased on July 7<sup>th</sup> (Figure 4. 2 b). In the spring, densities were always higher in OSR fields but similar in fallows, grasslands and roadsides (Figure 4. 2 a,c). In the summer, the pollen beetles were denser in the crops than in non-crops areas (Figure 4. 2c) and among non-crops areas they were denser in grassland than in roadsides and fallows (Figure 4. 2d).

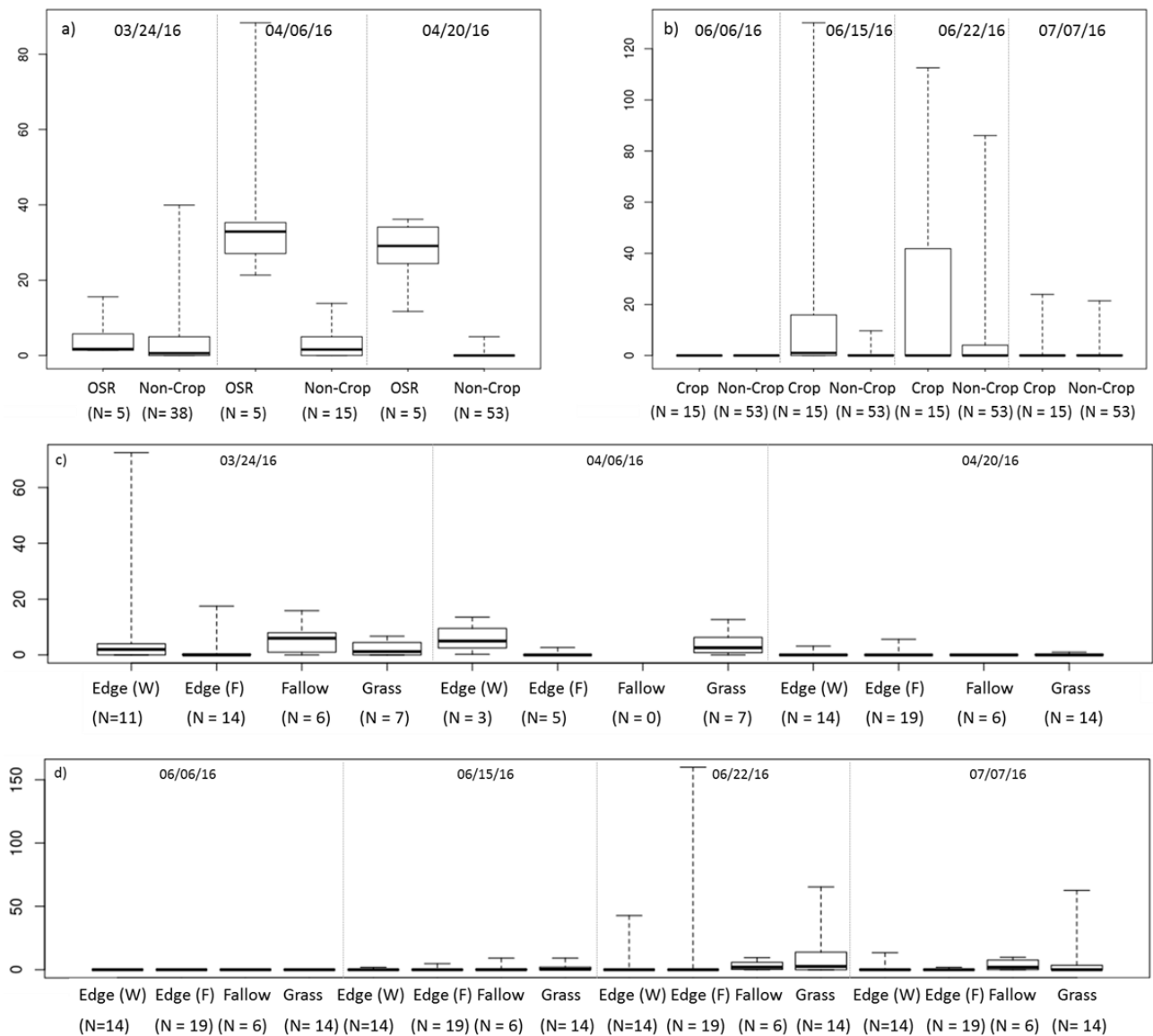


Figure 4. 2: Abundance of pollen beetles per m<sup>2</sup> and per date in a) Crop/non crop area during spring and b) in crop/OSR and non-crops areas during summer. Abundance per m<sup>2</sup> and date in c) different types of non-crop habitats during spring and d) in non-crop areas divided in fallow, grassland and edges during summer. Numbers of sites sampled are under the graph. Edge (W) = roadside of woodland, Edge (F) = roadside of field, Grass = grassland. The lower (0.025) and upper quantile (0.975) range of the mean of abundance of pollen beetles. The diagram also shows the median observation and the confident interval at 0.25 %.

First, in the spring, we determined if there were different densities of pollen beetles in the different habitats when we also considered the pollen beetles caught in the OSR fields (Tableau 4.2). The OSR fields had a high effect on the abundance of pollen beetles. Pollen beetles were denser in habitats with more woodlands and OSR fields in a buffer radius of 0.5 km (Tableau 4.2). We performed the same model discarding the OSR fields as surface sampled to determine if there were different densities of pollen beetles in the different habitats when they were not in OSR fields (Tableau 4. 2, N°4). In this new model, the factors of density corresponding to each

habitat have largely overlapped credible intervals. The surfaces of OSR and woodland were still significant and the last date became negatively significant.

Tableau 4. 2: Results of the GLM on the habitats (with OSR fields) and dates in the spring. Habitats and date were random effects. Values in bold when the 95% credible interval does not include 0.

	mean	2.50%	97.50%
Standardized Proportion of OSR in 0.5 km	<b>0.4</b>	<b>0.17</b>	<b>0.62</b>
Standardized Proportion of Wood in 0.5 km	<b>0.58</b>	<b>0.34</b>	<b>0.83</b>
Roadside (Woodland)	-0.72	-2.01	0.56
Roadside (Field)	-0.61	-1.89	0.64
OSR fields	<b>2.76</b>	<b>1.54</b>	<b>4.01</b>
Fallows	-1.02	-2.39	0.3
Grasslands	-0.36	-1.63	0.92
Date 1	0.37	-0.63	1.41
Date 2	0.63	-0.36	1.68
Date 3	-0.94	-1.95	0.03

### 3.2. Which flowers attract them in the spring?

To evaluate the effect of flowers on the abundance of pollen beetles, we performed the same model previously mentioned (Tableau 4.3, N°4) but added flowers variables or flowers characteristics as the total percentage of coverage by flowers in the quadrat, the number of flower species in the quadrat, the presence or absence of each flower species, the percentage of cover of each flower species and compared the WAIC of these models. The best model was which with the presence or absence of each flower species with a WAIC of 561 (Tableau 4.3, N°1, Tableau 4.4). The same variables had credible intervals not including 0 as in the model 4 without flowers. Five flower species had also a credible interval not including 0: the lamium, the ground ivy, the sinapis, the dandelion and the mahonia.

This model was not improved by the inclusion of the percentage of cover of all the flower species (WAIC of 650, Tableau 4.3 N°5). In this model, in addition to the associations already described, the anemone and the geranium were negatively associated with the abundance of pollen beetle. Moreover, the effect of lamium, mahonia, ground ivy and OSR surface were lost, keeping the effect of two yellow flowers.

The best model was neither improved by the grouping of the flower cover per quadrats (WAIC of 653) (Tableau 4.3 N°6) or by accounting uniquely the number of flower species per quadrat (WAIC of 638) (Tableau 4.3 N°3), suggesting that the specific flower species composition is more important than the general flower diversity.

## Chapitre 4 : Habitats des méligèthes hors des bois et des parcelles de colza

The same variables concerning habitat, date and surfaces of landscape elements had credible intervals not including 0 for the 3 models as for the model without flowers. The number of pollen beetle increased with the number of flower species [0,22, 0.97]. This suggests that the importance of richness of flowers for pollen beetles.

*Tableau 4.3: Summary of the models in the spring on the effect of the habitats, of surfaces of OSR and woodlands, of the flowers and of the characteristics of the flowers. The WAIC are also presented.*

N°	Season	Habitats	date	OSR surface	Wood surface	Flowers variable	flower charact	WAIC
1	Spring	without OSR	with date	with OSR	with wood	Pres/abs species	/	561
2	Spring	without OSR	with date	with OSR	with wood	/	Pres/abs	561
3	Spring	without OSR	with date	with OSR	with wood	Number of species	/	638
4	Spring	without OSR	with date	with OSR	with wood	/	/	649
5	Spring	without OSR	with date	with OSR	with wood	% cover each species	/	650
6	Spring	without OSR	with date	with OSR	with wood	% cover total	/	653
7	Spring	without OSR	with date	with OSR	with wood	/	% cover	655

Finally, to determine if the attractiveness of the flower species could be explained by their characteristics, we substituted the flowers by their characteristic. Again, the best model was based on the presence or absence of characteristics in the quadrat, with a WAIC similar to the model based on the flower species (WAIC of 561) (Tableau 4.3, N°2 Tableau 4.5). The model based on the cover associated with each characteristics was much worse (WAIC of 655.1). We found the funnel shape was not attractive and the lip shape attractive, which are both flowers shapes with nectar.

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Tableau 4.4: Result of the best model with the flowers in the spring. Values in bold when the 95% credible interval doesn't include 0.

	Mean	2.50%	97.50%
Anemone	-1.86	-5.93	1.52
Capsella	0.61	-2.02	3.89
Cardamine	1.95	-0.03	3.96
Ficaria	-0.18	-1.87	1.46
Broom	-1.77	-4.85	1.34
Geranium	-2.85	-6.25	0.15
Lamium	<b>2.67</b>	<b>1.57</b>	<b>3.79</b>
Ground ivy	<b>1.98</b>	<b>0.03</b>	<b>4.08</b>
Lycopsis	-1.71	-4.81	1.63
Mahonia	<b>3.67</b>	<b>1.45</b>	<b>6.17</b>
Mercurialis	-1.84	-6.37	3.16
Chickweed	-0.5	-2.01	1.18
Sinapis	<b>4.5</b>	<b>3.11</b>	<b>6.04</b>
Myosotis	-1.66	-5.67	1.73
Bellis	0.17	-0.94	1.31
Pansy	-1.34	-5.52	2.57
Dandelion	<b>3.25</b>	<b>2.34</b>	<b>4.25</b>
Primrose	-0.84	-3.2	1.39
Buttercup	1.02	-0.16	2.25
Ranunculus tuberosus	-0.71	-5.24	3.87
Potentilla	-1.58	-5.68	2.28
Senecio	-0.95	-2.44	0.53
Stellaria	-0.65	-3.55	2.44
Speedwell	0	-0.83	0.84
Violet	-1.31	-3.54	0.92
Viola alba	-0.86	-3.49	2.19
Standardized Proportion of OSR in 0.5 km	<b>0.58</b>	<b>0.12</b>	<b>1.07</b>
Standardized Proportion of Wood in 0.5 km	<b>1.57</b>	<b>0.95</b>	<b>2.2</b>
Roadside (Woodland)	-0.48	-1.92	0.74
Roadside (Field)	0.21	-0.92	1.6
Fallows	-0.84	-2.55	0.4
Grasslands	1.06	-0.06	2.55
Date 1	0.48	-1.03	1.95
Date 2	1.03	-0.46	2.57
Date 3	<b>-1.66</b>	<b>-3.22</b>	<b>-0.25</b>

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Tableau 4.5: Result of the best model with characteristics of flowers in the spring. Values in bold when the 95% credible interval doesn't include 0.

	Mean	2.50%	97.50%
Pollen	3.31	-0.33	7.25
Nectar	1.81	-1.48	5.75
Yellow	3.41	-0.34	7.31
White	-0.48	-1.69	0.76
Pink	0.71	-0.36	1.81
Blue	-1.15	-4.03	1.89
Purple	0.36	-2.55	3.07
Bell	-1.12	-5.44	3.34
Disk	-0.03	-0.93	0.92
Funnel	<b>-2.08</b>	<b>-4.05</b>	<b>-0.16</b>
FlagBlossom	-2.08	-4.59	0.67
Ray	0.59	-0.49	1.71
Lip	<b>1.06</b>	<b>0.15</b>	<b>1.98</b>
DiskRay	-0.74	-1.73	0.21
Standardized Proportion of OSR in 0.5 km	<b>0.67</b>	<b>0.27</b>	<b>1.1</b>
Standardized Proportion of Wood in 0.5 km	<b>1.13</b>	<b>0.69</b>	<b>1.65</b>
Roadside (Woodland)	-0.16	-1.08	0.44
Roadside (Field)	0.24	-0.28	1.29
Fallows	-0.25	-1.38	0.3
Grasslands	0.13	-0.43	0.93
Date 1	1.07	-0.67	2.77
Date 2	1	-0.74	2.74
Date 3	<b>-2.23</b>	<b>-4.07</b>	<b>-0.68</b>

### 3.3. Where are pollen beetles in the summer?

In the summer, unlike in the spring, habitats had clearly coefficients with significantly more than average pollen beetle in fields and significantly less than average pollen beetle in the roadside at the contact of the fields (Tableau 4.6) (Tableau4.7,N 7). The model with the buffer radius of 3 km for the proportion of woodlands and 2 km for the proportion of OSR was selected with a WAIC of 2598.9 (only 2 points of WAIC better than the second best model, Table S4.3). The coefficient of the proportion of OSR was positive. The date 2 was positively significant and the date 4 was negatively significant.

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Tableau 4.6: Results of the GLM on the habitats (with OSR fields) and dates in the summer. Habitats and date were random effects. Values in bold when the 95% credible interval doesn't include 0.

Variable	mean	2.50%	97.50%
(Intercept)	-0.21	-1.44	0.94
Proportion of OSR in 2 km	1.29	<b>0.94</b>	<b>1.63</b>
Proportion of wood in 3 km	0.24	-0.12	0.59
Roadside (Woodland)	0.05	-0.69	0.82
Roadside (Field)	-1.34	<b>-2.26</b>	<b>-0.51</b>
Fallows	-0.13	-0.87	0.66
Fields	0.93	<b>0.23</b>	<b>1.73</b>
Grasslands	0.51	-0.11	1.22
Date 1	0.94	-0.23	2.14
Date 2	2.05	<b>0.91</b>	<b>3.22</b>
Date 3	0.91	-0.22	2.12
Date 4	-3.91	<b>-5.61</b>	<b>-2.45</b>

Tableau4.7: Summary of the models in the summer on the effect of the habitats, of surfaces of OSR and woods, of the flowers and of the characteristics of the flowers. The WAIC are also presented.

N°	Season	Habitats	date	OSR surface	Wood surface	Flowers variable	flower charact	WAIC
1	Summer	without OSR	with date	with OSR	with wood	/	Pres/abs	2173.3
2	Summer	without OSR	with date	with OSR	with wood	Pres/abs species	/	2216.7
3	Summer	without OSR	with date	with OSR	with wood	Number of species	/	2402.6
4	Summer	without OSR	with date	with OSR	with wood	% cover each species	/	2494.3
5	Summer	without OSR	with date	with OSR	with wood	% cover total	/	2555.6
6	Summer	without OSR	with date	with OSR	with wood	/	% cover char.	2559.2
7	Summer	without OSR	with date	with OSR	with wood	/	/	2598.9

### 3.4. Which flowers attract them in the summer?

As we did for the spring, we added to this model the effect of flower species. The best model was which with the presence or absence of flower species (AIC of 2216) (Tableau4.7, N°2, Tableau 4.8). The same variables had credible intervals not including 0 than for the previous model, except that the fields lost their effect. In this model, 20 flower species had also credible intervals not including 0 and attracted pollen beetles. More flowers than in the spring were significantly correlated with the pollen beetles abundance, none were negatively correlated with the pollen beetle abundance. The same model but accounting for the percentage of cover of each species was worst (WAIC of 2494) and discarded 5 flower species (blackberry, buttercup, daisy, heracleum and lapsana) and added one species, bellflowers.

Tableau 4.8: Result of the best model with the flowers in the summer. Values in bold when the 95% credible interval doesn't include 0.



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Variable	Mean	2.50%	97.50%
Sinapis	<b>5.96</b>	<b>4.94</b>	<b>7.18</b>
Poppy	<b>5.2</b>	<b>4.41</b>	<b>6.01</b>
Salsify	<b>5.16</b>	<b>4.02</b>	<b>6.37</b>
Groundsel	<b>4.63</b>	<b>3.3</b>	<b>6.14</b>
Picris	<b>4.07</b>	<b>1.92</b>	<b>6.92</b>
Briar	<b>4.02</b>	<b>2.19</b>	<b>6.28</b>
Lapsana	<b>4.01</b>	<b>2.82</b>	<b>5.35</b>
Dandelion	<b>3.64</b>	<b>2.39</b>	<b>5.01</b>
Clover white	<b>2.56</b>	<b>1.5</b>	<b>3.73</b>
Hypericum	<b>3.53</b>	<b>1.67</b>	<b>5.77</b>
Campion	<b>3.52</b>	<b>2.07</b>	<b>5.19</b>
Blackberry	<b>2.73</b>	<b>1.16</b>	<b>4.53</b>
Buttercup	<b>2.54</b>	<b>1.16</b>	<b>4.2</b>
Clover violet	<b>2.53</b>	<b>1.59</b>	<b>3.53</b>
Daisy	<b>2.52</b>	<b>1.25</b>	<b>3.92</b>
Sonchus	<b>2.51</b>	<b>1.64</b>	<b>3.48</b>
Heracleum	<b>2.46</b>	<b>1.03</b>	<b>4.01</b>
Alfalfa	<b>2.06</b>	<b>1.28</b>	<b>2.85</b>
Knautia	<b>2</b>	<b>0.24</b>	<b>4.14</b>
Privet	<b>1.9</b>	<b>0.32</b>	<b>3.42</b>
Bellflower	1.12	-0.58	3.03
Thistle	2.31	-0.03	5.17
Cichorium	0.72	-2.04	3.88
Gaillum sp	-0.46	-3.39	2.86
Geranium	-0.08	-1.01	0.87
Geum	-0.63	-4.94	3.41
Lamium	-0.23	-4.84	4.41
Bindweed	1.69	-1.59	5.18
Lotus	2.33	-0.17	5.32
Matricaria	1.51	0	3.22
Chickweed	-0.07	-4.92	4.56
Myosotis	-0.23	-1.63	1.19
Nigella	2.08	-0.71	5.03
Orobanche	-1.76	-4.31	1.14
Bellis	0.95	-0.44	2.53
Eschscholzia	0.97	-1.33	3.61
Pansy	-1.19	-5.71	3.1
Potentilla	-0.86	-2.85	1.22
Polygonum	-0.42	-4.93	3.89
Alcea	-0.77	-5.15	3.43
Stellaria	-0.59	-4.56	3.87
Speedwell	-0.76	-3.53	2.29
Vicia	1.27	-0.44	3.25
Standardized Proportion of OSR in 0.5 km	<b>0.68</b>	<b>0.31</b>	<b>1.06</b>
Standardized Proportion of Wood in 0.5 km	-0.09	-0.43	0.27
Roadside (Woodland)	-0.06	-0.79	0.62
Roadside (Field)	-0.75	-1.84	0.02

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Fallows	0.14	-0.53	0.91
Fields	0.21	-0.42	0.99
Grasslands	0.39	-0.19	1.13
Date 1	0.64	-0.9	2.32
Date 2	<b>2.18</b>	<b>0.63</b>	<b>3.87</b>
Date 3	0.59	-1.02	2.36
Date 4	<b>-3.72</b>	<b>-6.54</b>	<b>-1.62</b>

As for the spring, we grouped the percentage of cover of all flower species, and the model was worst (WAIC of 2555) (Tableau4.7, N°5). Unlike in spring, the total percentage of cover was significant positive with a credible interval not including zero [0.07, 0.18] and the effect of roadside near fields was significant [-2.14, -0.57]. When we substituted the total percentage of cover by the number of flower species in the quadrat, the WAIC improved a lot (2402.6) changing the other parameters. The number of flowers per quadrat was significant with a credible interval not including zero [2.18, 3.04] as the grasslands [0.03, 1.4] and the woodlands [0.15, 0.82]. The same variables concerning habitat, date and surfaces of landscape elements had credible intervals not including 0 for the 3 models as for the model without flowers.

Finally, as for the spring, we determined the characteristics of flowers which were attractive for pollen beetles in summer. The best model was model performed with the presence or absence of these characteristics (WAIC of 2173.3) (Tableau4.7,N°1,Tableau 4. 9). The same landscape and date variables as for the best model were selected but none of the habitat variables. Some of the characteristics of flowers were attractive for pollen beetles: the presence of pollen, nectar, red and purple flowers and flowers with a flag shape. The model with the percentage of cover of the characteristics was worst with a WAIC of 2559.2 and had the same characteristics significant as the best model. None of the characteristics of flowers were significant.

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Tableau 4. 9: Result of the best model with characteristics of flowers in the spring. Values in bold when the 95% credible interval doesn't include 0.

Variabiles	mean	2.50%	97.50%
Pollen	<b>4.36</b>	<b>3.2</b>	<b>5.49</b>
Purple	<b>1.88</b>	<b>1</b>	<b>2.8</b>
Nectar	<b>1.94</b>	<b>0.85</b>	<b>3</b>
Red	<b>1.91</b>	<b>0.79</b>	<b>3.07</b>
Flag	<b>1.73</b>	<b>0.47</b>	<b>3.06</b>
Yellow	0.39	-0.76	1.49
White	0.64	-0.18	1.48
Pink	-0.16	-1	0.66
Blue	0.45	-1	1.88
Orange	-0.08	-1.96	2.11
Bell	-1.77	-3.6	0.4
Disk	0.06	-0.79	0.9
Funnel	-0.94	-2.38	0.58
Ray	0.53	-0.49	1.55
Lip	-0.92	-2.85	1.04
DiskRay	-0.98	-1.97	0.01
StandardizedProportionofOSRin0.5km	<b>0.93</b>	<b>0.53</b>	<b>1.32</b>
StandardizedProportionofWoodin0.5km	0.26	-0.11	0.61
Roadside(Woodland)	0.18	-0.54	1.03
Roadside(Field)	-0.74	-1.84	0.05
Fallows	-0.45	-1.38	0.31
Fields	0.46	-0.28	1.43
Grasslands	0.52	-0.1	1.35
Date1	0.57	-1.23	2.47
Date2	<b>2.38</b>	<b>0.68</b>	<b>4.24</b>
Date3	1.03	-0.7	2.87
Date4	<b>-4.35</b>	<b>-7.19</b>	<b>-2.12</b>

### 3.5. Which flowers attract them when some other flowers are present?

To determine the respective attractiveness of the flowers within a quadrat, we modelled the presence and absence of the pollen beetle on each flower species present in each quadrat, given the number of pollen beetle in the quadrat. We compared 6 models: with the percentage of cover for each flower species or with their presence-absence and with three increasingly weak priors (Figure 4.3). In the spring, *Sinapis arvensis*, *Taraxacum officinale* and *Ranunculus bulbosus* were significantly attractive. The *Veronica spp* and the *Cardamine pratensis* were lower attraction factors than average suggesting they might be repellent to pollen beetle within a quadrat. The 6 models had the same trends, in some cases, the model with the higher prior not significant compared to others.

In the summer, the *Knautia arvensis*, the *Leucanthemum vulgare*, the *Ligustrum*, the *Sinapis arvensis*, the *Rosa canina* were attractive for pollen beetles whereas the *Campanula rapunculus*, the *Geranium robertianum*, *Medicago sativa*, the *Myosotis spp* and *Vicia spp* had a repellent effect. Some species as *Taraxacum officinale* or *Rubus fruticosus* lost their significance when we use only the presence or absence of these flowers.

#### 4. Discussion

Our results showed that in both seasons pollen beetles were more abundant in quadrat sampled in crops. In the spring, the major part of the pollen beetles goes in oilseed rape fields. When pollen beetles were not in these fields, they were in fallows, grasslands and roadsides. In the summer, they can be found in majority in the weeds of crop fields and in minority in grasslands and roadsides of woodlands, before going in woodlands to overwinter. The pollen beetle were mainly attracted by flowers in these habitats in both seasons. Moreover, surrounding surfaces of cultivated fields with OSR and woodlands had a significant effect on pollen beetles number in the spring and only OSR in the summer, likely because they are origin or destination of the migration depending on the season.

In the spring, the pollen beetles were mainly found in oilseed rape fields. However, in the first dates, when oilseed rape was not flowering, we found a lot of pollen beetles in grasslands, roadsides and fallows. Nevertheless, in this landscape, the surfaces of fields cultivated by OSR are higher than surface of non-crop areas showing that pollen beetles were in majority in the OSR fields. In the summer, pollen beetles were found principally on weeds of fields and non-crops areas were also major pollen beetles feeding sites at the end of June. As for OSR fields in spring, the surfaces of fields with weeds were higher than surfaces of non-crop areas and host the major part of pollen beetles. Their abundance reached a peak rapidly in the middle of June. The decrease of abundance of pollen beetles could be a sign of their move into overwintering sites. Pollen beetles were known to begin to overwinter in the end of August (Williams & Free 1978). Here we suggest that in North-Western France they might overwinter since the beginning of July. These differences could be due to a shift in the flowering dates between England and France and between 1978 and now.

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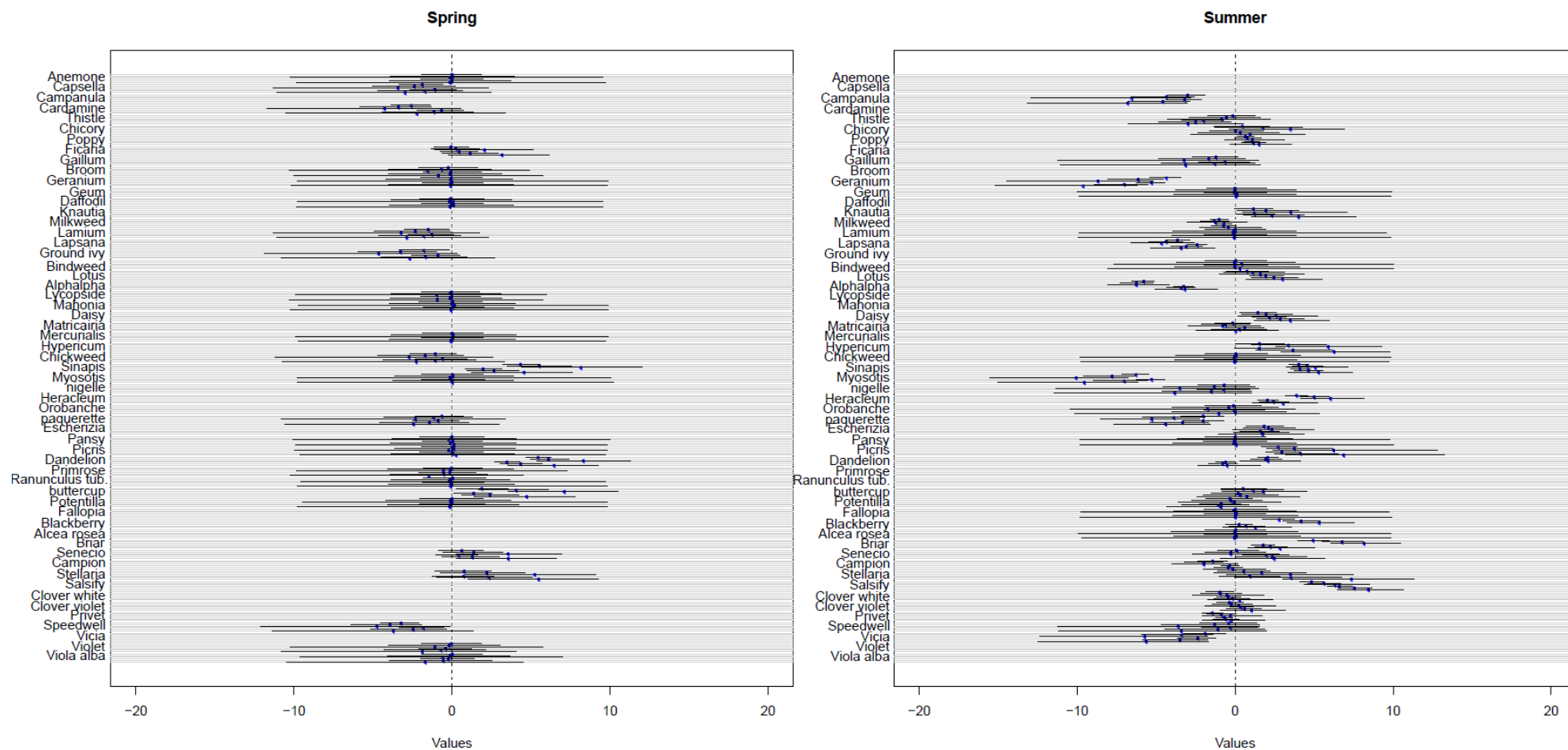


Figure 4.3 : Relative attractiveness of each flower species in spring and summer. For each line of each species, first three lines = models with percentage of cover of the flower species. The three other lines are models with the presence or absence of these flowers. The first lines of these categories were with the prior with  $sd = 1$ , the second, with  $sd = 2$  and the third, with  $sd = 5$ .

Here we showed that the grasslands were more attractive habitats for pollen beetles in both seasons than other non-crop habitats. Nevertheless, this attractiveness was highly different between each grassland and dates. This could be due to the mowing of the grasslands in the summer. The mowing of roadsides between week 25 and 27 could also explain why pollen beetles were more numerous in grasslands and fields edges than in roadsides. Fallows were also surfaces not mown but grassier, grass not being a resource for pollen beetles (Toivonen, 2016). Moreover, one previous study confirmed that pollen beetles can be found in field margins (Carrie et al., 2012).

In the spring, pollen beetles were more numerous in areas with a lot of OSR and woodlands in a buffer radius of 0.5 km. Pollen beetles emerge from woodlands (Rusch et al., 20012) and could only go into non crops areas when they leave woodlands and if they are at proximity. If OSR is not blossomed or in buds stage, they are attracted by its odor (Jonsson et al., 2007) but do not found resources and then could go to nearest non-crop areas, this would explain the effect of this small radius. These effects were robust as they kept their significance in the other models. In summer, pollen beetles were also more numerous in areas with a high proportion of surrounding OSR but within 2 km. In this season, pollen beetles emerge from OSR crops and seek pollen to stock up fat (Williams, 2010). They may have more pollen sources in this season and therefore can travel further. The proportion of woodlands was not significant, perhaps because their principal activity at this period was to search for feeding areas and not their overwinter sites.

We compared the model with only the habitat types, the date and the proportion of OSR and woodlands to models adding the total percentage of cover by flowers or adding the number of flower species per quadrat. In both seasons, the best model was the one with the number of flower species, showing that the richness is an important determinant of the attractiveness to the pollen beetle.

To determine if some flowers were more significant for pollen beetles, we performed the same model adding flowers in diverse modalities: the percentage of cover of all the flower species, and the presence or absence of each flowers. In the spring, the best model was which with only the presence or absence of flower. This models showed that *Taraxacum officinale* and *Sinapis arvensis* were both attractive for the pollen beetle. This first species is known to attract pollen beetles (Free & Williams, 1978, (Taimr et al., 1967), and the second is a brassicacea known to attract them too (Kaasik et al., 2014). The *Mahonia aquifolium* was also attractive confirming

the effect of the color yellow on pollen beetles. The lamium and the ground ivy were also attractive in this model but were discarded in the model with the percentage of cover of each flower species and in the model a factor of attraction of each flower species. Their effect seems to be unclear. Other flower species were significant in other models but not in the best model, suggesting also a weak or uncertain effect of these flowers.

In the summer, a wider variety of flowers were found to attract pollen beetles with the best model with the flowers, which is the model with the presence or absence of flower species. The majority of these species had pollen and could explain their positive effect on pollen beetles. In this period, they eat pollen to stock up fat and thus increase their survival during overwintering (Cook et al., 2004). This behavior is well known in insects that diapause (Hahn & Denlinger, 2007), and pollen beetles have a high mortality rate in winter (Hokkanen, 2000). The other flower species had nectar and perhaps pollen beetles need nectar at this period. Moreover, the *Knautia arvensis*, the *Leucanthemum vulgare*, the *Ligustrum*, the *Sinapis arvensis*, the *Rosa canina* were found to be attractive for pollen beetles. These flower species detected by these 3 models were the most relevant for pollen beetles in summer.

The adult pollen beetles were found on 31 plant species out of the 58 plant species sampled, on 13 species in spring and 25 in summer. In another study, pollen beetles were found on only *Taraxacum officinale* in the spring and on 34 plant species in summer (Free & Williams, 1978) but only 5 species are in agreement with our plant species which were *rosa sp*, *rubus fruticosus*, *Senecio sp*, *Sinapis arvensis* and *Taraxacum officinale*. In Ouvrard et al. (2016), they found pollen beetles in the summer in 41 plant species from 9 families from a wide range of colors and shapes. Only 10 plant species were the same as ours. These differences highlight the wide range of flowers in which we can find pollen beetles.

Odors and colors of flowers are known to be a determinant of pollen beetles attraction by flowers (Jonsson et al., 2007). In our study, in spring, we found that pollen beetles were not attracted by funnel shape flowers, which are often flowers with absence of pollen. They also were attracted by lip shape flowers, this is surprising as this kind of flowers have difficult access to the pollen for insects (Klotz et al., 2002). As pollen beetles are small insects of 2 mm, they could access at the pollen of these shapes. In the summer, the model with characteristics of flowers was the best overall. The pollen beetles seem to be attracted by flowers with presence of pollen, nectar, which were red or purple flowers and flowers with a flag shape. The pollen and nectar confirm the selection of some flowers in other models. The red color seems to be

linked to poppy in which we found a lot of pollen beetles and the purple for a variety of flowers with this color.

In the spring, all these results on color are in agreement with the literature showing that pollen beetles are attracted by oilseed rape flowers (Cook et al., 2002) and have higher preference for oilseed rape odor than individuals we can find in the summer. Pollen beetles were not sensible to color without odor stimuli (Jonsson et al., 2007). Another study, with oilseed rape petals of diverse colors, showed that pollen beetles (in the spring) preferred yellow and white than red and blue petals (Cook et al., 2013). In our study, we found a stronger effect of yellow flowers species in the spring than in summer. In spring, pollen beetles seek oilseed rape fields, they are attracted by these flowers, and so for yellow, if they found other flowers in their way they eat pollen or nectar on them. In summer, they just seek sources of pollen, and so do not account for flowers color.

In our study, a limitation was that the selection of models with the presence or absence of flowers and not of the percentage of cover for these flowers could show the importance of only the presence of species. Nevertheless, here, the percentage of cover was estimated in situ and was slightly different between species and between sites. A homogenization of these data is needed. Further analysis could compare pollen beetles abundance by directly counting pollen beetles by flowers to specify the role of each kind of flowers. We did not account for ornamental flowers in gardens, this surface could be compared to others habitats. We found buffer radius of 2 and 3 km as the best buffer sizes in summer, our study site was of 6 by 8 km, the buffers covered a large part of our site. Moreover, this site was in a landscape with a lot of non-crops areas, our pollen numbers could be compared to pollen beetles numbers in open landscapes with few non-crops areas.

In this study, we showed that the main habitats for pollen beetles were oilseed rape fields in the spring and other fields in the summer. The non-crop habitat could have a role on the feeding of pollen beetles to a lesser extent. A solution could be to mow these non-crop areas before the flowering of oilseed rape to prevent pollen beetles attacks, and mow them at the peak of emergence, in June. However, the non-crops areas such as grasslands, roadsides, fallows and woodlands are known to be major habitats for a wide range of natural enemies (Bianchi et al., 2006) and pollinators (Holzschuh et al., 2008). The ambivalence of these areas, useful for natural enemies and pests (Bianchi et al., 2006, Van Emden, 1964) implies difficulties in their management. The weeds in fields are source of pollen for *B. aeneus*. A best management of



these weeds as their removal in spring could reduce populations of pollen beetles. However, these flowers are also feeding sources for natural enemies (Franke et al., 2008). Weeds are also sources of pollen and nectar for bees and other pollinators (Bretagnolle & Gaba, 2015). These results highlights the difficulties to manage non-crops areas accounting for pest and for their natural enemies.

### **Acknowledgements**

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## Supplementary material

Table S4.1: Characteristics of sampled flowers. Pollen and nectar: none, present or plenty.

Name	Color	Pollen	Nectar	Shape	PB in spring	PB in summer	Flowering period
<i>Alcea rosea</i>	pink	present	present	disc	no	no	May-September
<i>Capsella bursa-pastoris</i>	white	none	present	disc	no	no	May-October
<i>Cardamine pratensis</i>	white	none	present	disc	no	no	April-May
<i>Centaurea cyanus</i>	blue	none	present	disc	no	no	May-July
<i>Cichorium intybus</i>	purple	none	present	disc	no	no	July-September
<i>Fallopia japonica</i>	white	none	present	bell	no	no	July-September
<i>Galium spp</i>	white	none	none	disc	no	no	July-September
<i>Geranium robertianum</i>	pink	none	plenty	disc	no	no	June-September
<i>Geum urbanum</i>	yellow	present	present	disc	no	no	May-July
<i>Glechoma hederacea</i>	pink	none	present	lip	no	no	March-May
<i>Hypericum perforatum</i>	yellow	plenty	none	ray	no	no	June-October
<i>Lamium purpureum</i>	pink	none	present	lip	no	no	March-December
<i>Lotus corniculatus</i>	yellow	none	present	flag	no	no	March-July
<i>Lycopus arvensis</i>	blue	none	present	funnel	no	no	June-September
<i>Mercurialis spp</i>	green	none	present	no	no	no	April-June
<i>Myosotis spp</i>	blue	none	present	funnel	no	no	June-August
<i>Narcissus jonquilla</i>	yellow	plenty	plenty	bell	no	no	January-March
<i>Orobanche purpurea</i>	purple	none	present	lip	no	no	June-July
<i>Primula veris</i>	yellow	plenty	present	funnel	no	no	April-May
<i>Ranunculus ololeucos</i>	white	plenty	plenty	disc	no	no	March-June February- November
<i>Stellaria media</i>	white	none	present	disc	no	no	

## Chapitre 4 : Habitats des méligèthes hors des bois et des parcelles de colza

<i>Stellaria spp</i>	white	present	present	disc	no	no	February- November
<i>Veronica spp</i>	blue	none	present	lip	no	no	May-October
<i>Vicia spp</i>	purple	none	present	flag	no	no	May-July
<i>Viola alba</i>	white	none	present	flag	no	no	February-April
<i>Viola sylvestris</i>	purple	none	present	flag	no	no	March-June
<i>Viola tricolor</i>	purple	none	present	flag	no	no	May-September
<i>Anemone nemorosa</i>	white	plenty	none	disc	yes	no	March-May
<i>Cytisus scoparius</i>	yellow	plenty	none	flag	yes	no	March-June
<i>Ficaria verna</i>	yellow	plenty	none	ray	yes	no	March-May
<i>Mahonia aquifolium</i>	yellow	present	present	discray	yes	no	March-April
<i>Ranunculus ficaria</i>	yellow	plenty	plenty	disc	yes	no	March-May
<i>Ranunculusbulbosus</i>	yellow	present	present	disc	yes	no	April-July
<i>Bellis perennis</i>	yellow	present	present	discray	no	yes	April-June
<i>Campanula rapunculus</i>	purple	present	present	bell	no	yes	May-July
<i>Convolvulus arvensis</i>	white	present	present	funnel	no	yes	May-October
<i>Eschscholzioideae</i>	orange	plenty	none	disc	no	yes	May-August
<i>Knautia arvensis</i>	pink	present	present	discray	no	yes	June-October
<i>Leucanthemum vulgare</i>	yellow	present	present	discray	no	yes	May-July
<i>Ligustrum spp</i>	white	present	present	funnel	no	yes	June-July
<i>Matricaria spp</i>	yellow	present	present	disc	no	yes	July-October
<i>Medicago sativa</i>	pink	none	present	flag	no	yes	July-August
<i>Nigella damascena</i>	blue	present	present	disc	no	yes	July-August
<i>Papaver rhoeas</i>	red	plenty	none	ray	no	yes	May-July
<i>Rosa canina</i>	pink	plenty	none	disc	no	yes	April-July
<i>Rubus fruticosus</i>	white	plenty	plenty	disc	no	yes	June-August
<i>Sambucus spp</i>	white	plenty	present	disc	no	yes	May-June
<i>Silene spp</i>	white	present	plenty	funnel	no	yes	May-September
<i>Silybum marianum</i>	purple	present	present	disc	no	yes	June-August
<i>Trifolium pratense</i>	purple	none	present	flag	no	yes	May-September
<i>Trifolium repens</i>	white	none	present	flag	no	yes	June-August
<i>Lapsana communis</i>	yellow	present	present	ray	yes	yes	June-August
<i>Picris echioides</i>	yellow	present	present	ray	yes	yes	June-September
<i>Senecio spp</i>	yellow	present	present	discray	yes	yes	March-July
<i>Sinapis arvensis</i>	yellow	plenty	present	disc	yes	yes	June-September
<i>Sonchus arvensis</i>	yellow	present	present	ray	yes	yes	July-August March-
<i>Taraxacum officinale</i>	yellow	plenty	present	ray	yes	yes	November
<i>Tragopogon pratensis</i>	yellow	present	present	ray	yes	yes	May-August

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Table S4.2: Mean of the percentages of cover per quadrats per sites. R = roadside, F = fallow, G = Grassland and C = Crop.

Site	Spring			Summer			
	03/24/16	04/06/2016	04/20/16	06/06/2016	06/15/16	06/22/16	07/07/2016
R01	0.30	NA	0.02	0.00	0.00	0.00	0.00
R02	30.10	NA	10.30	0.00	0.00	0.00	5.48
R03	10.20	NA	0.74	0.00	1.06	15.24	0.00
R04	16.50	NA	5.06	0.00	0.02	0.00	0.00
R05	0.50	NA	10.60	0.00	0.00	0.00	0.00
R06	6.30	NA	9.52	0.00	0.00	0.00	0.00
R07	0.10	NA	5.22	0.00	0.10	10.64	0.08
R08	5.20	NA	8.30	0.00	0.02	0.00	0.00
R09	38.00	NA	12.48	0.00	0.00	0.00	0.00
R10	10.00	NA	3.24	0.40	0.22	4.70	1.16
R11	10.20	NA	4.52	0.76	0.32	0.18	0.26
R12	NA	0.11	3.72	0.14	2.38	35.90	19.90
R13	NA	0.20	8.20	0.04	0.34	2.08	0.04
R14	NA	1.30	5.84	0.00	0.00	0.00	0.00
R15	NA	25.20	8.24	0.00	0.00	0.00	0.00
R16	NA	1.00	0.06	0.04	0.00	0.00	0.00
R17	NA	0.10	0.00	0.04	0.00	0.00	0.00
R18	NA	0.10	0.00	0.00	0.00	0.00	0.00
R19	NA	85.20	10.98	0.00	0.00	0.00	0.00
R20	0.00	NA	2.22	0.00	0.82	0.32	1.36
R21	0.00	NA	0.08	0.00	0.00	0.00	0.00
R22	0.00	NA	0.02	0.00	0.00	0.00	0.00
R23	0.00	NA	7.22	0.00	0.00	0.00	0.00
R24	0.00	NA	0.22	0.02	0.14	46.00	5.02
R25	25.00	NA	11.60	0.86	18.00	0.20	0.14
R26	0.10	NA	0.20	9.28	0.00	0.00	0.00
R27	0.00	NA	0.00	0.00	0.00	0.00	0.00
R28	25.10	NA	9.08	0.04	0.00	0.00	0.00
R29	42.50	NA	1.32	0.00	0.00	0.00	0.00
R30	3.10	NA	42.00	0.04	0.00	0.00	0.00
R31	20.30	NA	22.70	0.00	0.00	0.00	0.00
R32	0.10	NA	1.00	0.00	0.00	0.00	0.00
G01	NA	0.00	8.40	6.12	6.46	7.10	4.12
G02	NA	0.00	19.34	1.14	1.98	2.52	16.84
G03	NA	0.26	4.80	0.02	6.02	0.30	0.00
G04	41.30	10.28	9.34	8.12	23.46	31.08	21.10
G05	0.70	NA	6.80	0.02	1.02	0.20	0.00
G06	0.20	NA	12.46	0.00	0.00	0.00	0.00
F01	NA	0.00	0.14	0.00	0.04	0.64	15.50
G07	NA	18.42	43.62	0.54	0.60	0.00	0.08
G08	NA	8.44	0.20	0.00	0.00	0.00	0.00
F02	0.30	NA	1.28	18.58	26.06	31.50	19.06
G09	NA	0.30	0.03	0.04	0.12	0.08	1.76
G10	4.42	NA	54.10	1.08	1.48	4.00	18.50
G11	0.11	NA	2.32	0.22	0.00	0.00	0.00

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F03	0.30	NA	4.22	0.08	0.00	0.00	0.00
G12	0.30	NA	0.44	0.04	2.00	7.00	0.00
G13	0.30	NA	2.24	0.44	5.08	3.04	0.30
F04	0.40	NA	1.22	1.26	3.44	7.50	32.58
F05	76.10	NA	28.36	0.00	0.00	0.00	0.00
F06	1.21	NA	21.20	1.64	0.08	2.36	2.24
G14	0.11	NA	1.42	0.04	0.08	0.26	0.00
C01	NA	NA	NA	NA	19.52	15.66	22.14
C02	NA	NA	NA	NA	0.00	0.00	0.04
C03	NA	NA	NA	NA	0.00	0.00	0.22
C04	NA	NA	NA	NA	22.06	5.54	0.00
C05	NA	NA	NA	NA	11.86	0.00	0.00
C06	NA	NA	NA	NA	0.00	0.00	0.00
C07	NA	NA	NA	NA	4.20	3.22	0.10
C08	NA	NA	NA	NA	0.02	0.02	0.00
C09	NA	NA	NA	NA	0.04	0.00	0.00
C10	NA	NA	NA	NA	0.00	0.00	0.04
C11	NA	NA	NA	NA	1.20	1.26	1.42
C12	NA	NA	NA	NA	4.76	2.32	0.16
C13	NA	NA	NA	NA	0.00	0.00	0.00
C14	NA	NA	NA	NA	0.00	0.00	0.00
C15	NA	NA	NA	NA	0.04	0.08	0.02

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Table S4.3:

OSR proportion	Wood proportion	Spring	Summer
0.5	0.5	648.9	2630.9
0.5	1.0	662.0	2635.1
0.5	1.5	670.4	2636.3
0.5	2.0	670.2	2632.1
0.5	2.5	669.4	2633.0
0.5	3.0	670.2	2628.8
1.0	0.5	664.1	2639.3
1.0	1.0	666.9	2639.9
1.0	1.5	670.4	2640.4
1.0	2.0	669.8	2640.0
1.0	2.5	668.5	2641.3
1.0	3.0	667.7	2641.0
1.5	0.5	660.1	2635.5
1.5	1.0	664.5	2628.0
1.5	1.5	664.5	2630.6
1.5	2.0	664.1	2630.3
1.5	2.5	664.0	2632.6
1.5	3.0	665.2	2630.0
2.0	0.5	658.1	2604.9
2.0	1.0	662.4	2601.7
2.0	1.5	666.4	2603.8
2.0	2.0	666.3	2602.9
2.0	2.5	665.4	2600.3
2.0	3.0	664.7	2598.9
2.5	0.5	656.0	2636.0
2.5	1.0	660.3	2641.3
2.5	1.5	661.6	2642.3
2.5	2.0	660.2	2639.2
2.5	2.5	659.3	2642.1
2.5	3.0	658.8	2643.3
3.0	0.5	657.7	2645.3
3.0	1.0	664.7	2650.6
3.0	1.5	671.8	2650.8
3.0	2.0	670.6	2650.4
3.0	2.5	670.5	2650.8
3.0	3.0	670.0	2651.1







## Chapitre 5

Développement et utilisation de  
marqueurs microsatellites pour améliorer  
les connaissances sur un parasitoïde d'un  
ravageur du colza, *Tersilochus*



## **Chapitre 5 : Développement et utilisation de marqueurs microsatellites pour améliorer les connaissances sur un parasitoïde d'un ravageur du colza, *Tersilochus heterocerus***

Les parties précédentes ont affiné les connaissances sur la dynamique des populations du méligèthes et leur écologie. A partir de ce chapitre nous allons nous concentrer sur le parasitoïdes de méligèthes, *Tersilochus heterocerus* (Thomson), de manière symétrique aux chapitres sur les méligèthes : structuration génétique puis dispersion et rôles des habitats semi-naturels.

Ainsi, comme pour les méligèthes, pour mettre en lumière leur structure génétique, nous avons mis au point des marqueurs microsatellites, l'objectif étant de comparer la structure génétique des méligèthes à celle de leurs parasitoïdes. Des parasitoïdes provenant de cinq échantillons de population en Europe: Autriche, Suède, Estonie, France (départements de l'Eure et Ille & Vilaine) et de deux générations successives de *T. heterocerus* collectés dans de nombreuses parcelles de colza dans l'Eure ont été analysés avec ces nouveaux marqueurs pour révéler la structure génétique entre populations et des liens de parenté entre individus.

Cette partie fera l'objet d'un article qui sera publié dans une revue à comité de lecture en génétique et s'intitulera « **Using microsatellite markers to improve knowledge of a crop pest parasitoid, *Tersilochus heterocerus*** ».



## **Using microsatellite markers to improve knowledge on the dispersal of a crop pest parasitoid, *Tersilochus heterocerus***

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### **Abstract**

One of the possible ways to reduce pesticide use is to improve the pressure from natural enemies on insect pests. Parasitoids are important biological control agents in agroecosystems. *Tersilochus heterocerus* (Thomson) is one of the most abundant and widespread parasitoid species of the pollen beetle (*Brassicogethes aeneus* F.) but nothing is known about the genetic structure of its populations. To shed light on their genetic patterns, we developed microsatellite markers. Sixteen out of the 40 designed loci turned out to be polymorphic on *T. heterocerus* and were relevant for population genetic studies. The 16 loci cross-amplified and seemed to be polymorphic in some other Ichneumonidae species such as *Phradis interstitialis*. Parasitoids from five population samples in Europe: Austria, Sweden, Estonia, and France (Eure and Ille & Vilaine departments) and from two successive generations of *T.heterocerus* collected in many oil seed rape fields in the Eure department were analyzed at these 16 loci to reveal the genetic structure between populations and sibship between individuals.

The genetic variability was similar among population samples except for the Swedish population. The genic differentiation was significant over population samples in Europe, which were structured in two clusters. We found similar proportions of full sibs and half sibs among the population samples in Europe. The effective population sizes were similar among the population samples and between generations for a population from the Eure.

## 1. Introduction

Parasitoids wasps are one of the main biological control agents in agroecosystems (Hawkins et al 1997). *Tersilochus heterocerus*, is one of the main parasitoids of pollen beetles, *Brassicogethes aeneus* (formerly named *Meligethes aeneus*), a pest of oilseed rape (OSR) (Ulber et al., 2010). The female parasitoid lays his eggs into pollen beetles larva, the larva drop and pupate in the soil. Then the parasitoid egg develops within the pollen beetle larvae and overwinter within it in the soil until the following spring (Williams, 2006).

*T. heterocerus* is impacted by the landscape structure, indeed this wasp is more abundant in complex sites with high proportion of non-crops areas (Thies & Tschardtke, 1999) such as woodlands and grasslands (Rusch et al., 2012a). The effect of landscape complexity has been explained by difficulties for parasitoids to move in high open fields (Thies et al., 2008). It can also be explained by the number of flowers that can be found in semi-natural habitats there, which are sources of nectar and pollen for parasitoid young adults (Rusch et al., 2013). Indeed, pollen beetles use woodlands for overwintering (Rusch et al., 2012b) and the grasslands as feeding sites (Juhel et al., 2017).

*T. heterocerus* are to some extent difficult to observe in the fields (Bernays & Chapman, 1994), but genetics tools might improve our knowledge on *T. heterocerus* (MacDonald & Loxdale, 2004). The microsatellite loci are codominant and highly variable genetic markers, two important criteria for assessing genetic variability in populations and relatedness between individuals (Sunnucks, 2000). These markers have already been used to study the population structure of parasitoids of crop pests, particularly of introduced species (Hufbauer et al., 2004). They have also been used on endemic parasitoids to analyze their breeding strategies (Tentelier et al., 2008) or their population structure (Jourdie et al., 2010).

In the present study, we developed microsatellite markers for a parasitoid *Tersilochus heterocerus* of the main OSR pest, *Brassicogethes aeneus*. We tested the efficiency of this newly developed set of microsatellite loci in describing the genetic structure of this wasp in Europe. Moreover we searched to measure the dispersal of this species with these markers using sibship analysis.

## 2. Materiel and methods

### 2.1. Microsatellite development

To develop and characterize markers, *T. heterocerus* were sampled, in the soil of oilseed rape crops in the Eure French department, in autumn 2014. Total DNA of the 21 *T. heterocerus* sampled, was extracted following the DNeasy® Blood & Tissue Kit (Qiagen) DNA extraction protocol modified as follows: 180 µl of Buffer ATL was added to each sample, then they all were ground using 3.15 mm steel beads on a 1600 MiniG (Spex® SamplePrep) tissue homogenizer at 1500 strokes/min for 1 minute. Extraction was then continued following the "Animal Tissues Spin-column Protocol" (DNeasy® Blood & Tissue Kit Handbook, p. 28-30). Microsatellite library development was based on the pool of the DNA of the 15 parasitoids and performed by GenoScreen (Lille, France), involving Roche 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries (Malausa et al. 2011). A total of 40 microsatellite markers were selected out of the 5,368 microsatellite sequences available based on the number of repeated motifs, the size of amplified fragments and the presence non-repeated sequences flanking microsatellite array longer than 20 pb each. Specific primer pairs were designed for each marker using Primer3 (Rozen & Skaletsky 2000).

PCR amplifications were attempted at these loci on six individuals. Each forward specific primer was conjugated with a 5'-GTTGTAAAACGACGGCCAGT-3' M13-tail at its 5' end as in Juhel et al., (2017b). 21 microsatellite loci out of 40 amplified on the six *T. heterocerus* specimens tested. These loci were chosen to estimate the polymorphism in a population of 25 individuals collected in one field of oilseed rape crop in France (Ille & Vilaine) (location 1, Figure 1). The number of alleles per locus, heterozygote proportion ( $H_o$ ) and gene diversity ( $H_E$ ) were estimated and departure to Hardy-Weinberg equilibrium were tested using exact test implemented in Genepop, version 4.2.2 (Rousset, 2008). We selected the polymorphic loci with less than 10% of null allele and that were at HWE. The selected microsatellite loci were combined in PCR multiplex, labelling each forward primer with a fluorescent dye at their 5'-end, either 6-FAM (6-carboxyfluorescein), or HEX (hexachloro-fluoresceine), or TAMRA (carboxy-tetramethyl-rhodamine), or ATTO 565 (Rhodamine dyesclass) (Table S1). These loci were also tested on five *Phradis interstitialis* individuals to check for interspecific cross-amplification.



## 2.2. Study sites and insect sampling

First, *T. heterocerus* adults were caught by beating in one OSR field per country in Estonia, Sweden, Austria and France (Ille & Vilaine) in 2015 (Figure 5.1A). Second, both *T. heterocerus* adults and *B. aeneus* larvae were sampled in 97 OSR fields in May 2016 by beating 15 OSR plants per field in the Eure department, France (Figure 5.1B). *T. heterocerus* adults from one of these fields were analyzed with the four other population samples in Europe to describe the population genetic structure of this species. In this region, two parasitoids species can be observed *T. heterocerus* and *Phradis interstitialis*, according to Osborn (1960). The genus *Tersilochus* have 16 antennal segments with the fourth shorter than the third or the fifth whereas genus *Phradis* have 15-16 antennal segments without length differences. The forewing vein 2-m-cu leaves vein M at the junction of veins forming the ariolet for *Phradis* whereas the forewing vein 2-m-cu leaves the vein M after the junction for *Tersilochus*. Finally, we distinguished for *T. heterocerus* males and females: the ovipositor of *T. heterocerus* female is particular, smoothly curved, clearly toothed dorsally and shallowly toothed ventrally. The ovipositor is visible, the females were distinguished from males with this detail. All the *T. heterocerus* were used in the sibship analysis described below

We identified the pollen beetles larvae that were parasitized: the larvae are sufficiently transparent to see the dark eggs under a binocular magnifier (Osborn 1960). We then extracted *T. heterocerus* eggs from these larvae. The adult parasitoids were found in 39 of the 96 fields (Tableau 5.1).

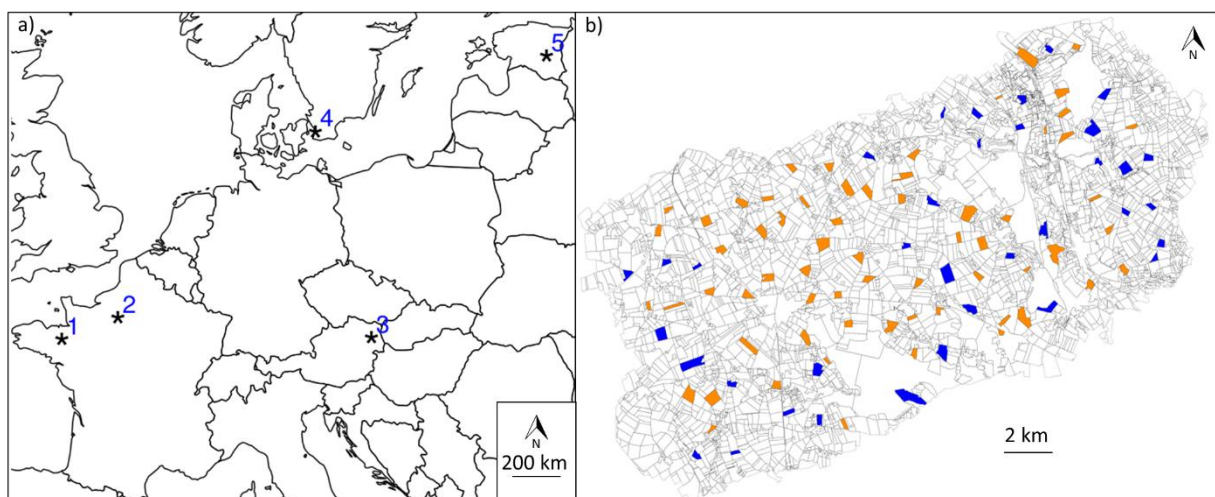


Figure 5.1: Geographical positions of the a) five *T. heterocerus* European population samples b) of the fields sampled with adults parasitoids (Orange) and without adults parasitoids (Blue). 1: France (Ille & Vilaine), 2: France (Eure), 3: Austria, 4: Sweden, 5: Estonia

### 2.3. Population structure and diversity analysis

#### 2.3.1. Microsatellite analysis

DNA extractions were performed in a 96-well format plate as in Juhel et al. (2017b). First, tissues of each parasitoid adults were ground in 50  $\mu$ L of H<sub>2</sub>O using a 2 mm steel bead using a 1600 MiniG (Spex® SamplePrep) homogenizer at 1500 strokes/min for 30 seconds. Second, tissues were digested at 56°C for 14 hours in a 100  $\mu$ l solution including 10% Chelex® 100 (Biorad) and 3% proteinase K using a Mastercycler thermocycler (Eppendorf). The tissue digestion was stopped by a final thermocycler step of 30 min at 98°C. Finally, the supernatant of this solution was used as a DNA template for the PCR reactions. A simplified protocol was applied for the eggs: we discarded the ground step and only added 30  $\mu$ l of the Chelex/proteinase K solution. PCR amplifications were done as in Juhel et al., (2017b).

#### 2.3.2. Analysis of the genetic data

A total of 1 111 parasitoids individuals were genotyped at the selected microsatellite loci and we analyzed the genetic diversity in the 5 European population samples (Table 1). We calculated at each locus in each population sample, the proportion of heterozygote,  $H_o$ , in female parasitoids (males being haploids) and gene diversity,  $H_E$ , based on allele frequencies estimated on both males and females. Allelic richness in each population sample was estimated using a rarefaction method (Hurlbert, 1971), as implemented in the HP-RARE program (Kalinowski, 2005). As we had populations with few females, we did not performed FST tests (Weir & Cockerham 1984). Exact test for departure from the Hardy-Weinberg equilibrium (HWE), genic differentiations and linkage disequilibrium (LD) between pairs of loci were performed using Genepop version 4.2.2 (Rousset et al 2008).

Tableau 5.1: Number of sampled OSR fields and number of genotyped males and females in each population samples.

Population	Instar	OSR field	Females	Males	Total
Austria	Adult	1	10	10	20
Estonia	Adult	1	10	12	22
France (Eure)	Adult	1	1	25	26
France (Ile & Vilaine)	Adult	1	25	/	25
Sweden	Adult	1	/	18	18
France (Eure total)	Adult	1	9	119	128
France (Eure)	Eggs	39	40	52	92

To detect clusters among the sampled parasitoids, we used the Bayesian clustering method implemented in Structure version 2.3.4 (Pritchard *et al.*, 2000). We selected the optimal number of groups  $K$  in which individuals should be assigned by testing the likelihood of models with values of  $K$  from 1 to 10. For each model, we performed 10 runs of 500 000 iterations after a ‘burn-in’ period of 200 000 iterations. Individual assignments were computed assuming admixture among the  $K$  groups. As recommended by Wang (2016), the geographical locations of the sampled individuals and non-equal contribution of the  $K$  sources to the admixture were used as priors for the structuration. We performed this analysis on the males and females from the five European populations.

### 2.3.3. *Statistics for sibship assignment*

To determine if there were similarities between European populations in terms of proportion of full sibs and half sibs per field. This analysis was conducted using the program COLONY version 2.0.6.3 (Jones and Wang 2010). Sibship assignments were based on likelihood ratio tests. The mating behavior in *T. heterocerus* is not known; we supposed that both sexes are polygamous. We used models accounting for haplo-diploidy. We used the Full-Likelihood and Pair-Likelihood combined method (FPLS). We used the percentage of null alleles estimated at each locus in the reference population from Ile & Vilaine to set up genotype errors. We did not update allele frequency and did not use the sibship scaling. All other parameters were set as default. We ran the model 10 times with a different seed and kept only full sibs and half sibs (maternalship and paternalship) pairs found in each of the 10 replicates. We performed these models on diverse data sets with males and females. We performed 1) kinship assignments among parasitoids in an OSR field to estimate  $N_e$  (independently in the five European population samples), 2) kinship assignment among parasitoids from several fields in the Eure department to estimate dispersal between fields, and 3) parentage assignment of parasitoid eggs.

The program COLONY permit also to estimate the effective population size ( $N_e$ ) based on the sibship assignments (Wang, 2009). We estimated  $N_e$  with this method for the 7 different models of assignment, based on the full sibs and half sibs (paternal ships and maternal ships) kept by the 10 models.

### 3. Results

#### 3.1. Design and selection of microsatellite markers

We analysed the genetic variability at each microsatellite locus on 25 *T. heterocerus* females from the Ille & Vilaine population used as reference to develop the markers. Among the 21 loci genotyped in this population, three were monomorphic (*Th-AFT3B*, *Th-B95QV*, *Th-cons698*) and two did not amplify in several individuals (*Th-B7116*, *Th-ARXDT*). The 16 remaining loci showed a large range of polymorphism (2 to 7 alleles per locus): proportion of heterozygotes ranged from 0.14 to 0.77 and gene diversity ranged from 0.12 to 0.73 (Tableau 5.2). None deviated from the HWE or had a frequency of null alleles lower than 10 %. Cross-amplifications of these 16 *T. heterocerus* microsatellite loci on *P. interstitialis* were successful but few loci were polymorphic in this species (Table S5.2).

Tableau 5.2 : Characterization of 16 polymorphic microsatellite loci in *Tersilochus heterocerus*. Locus Name, forward (F) and reverse (R) primer sequences, repeat in sequenced clone, sizes of the PCR products, number of alleles (Na), proportion of heterozygotes (Hp) and gene diversity (Hd), frequency of null alleles, expected and observed heterozygosity ( $H_e$  and  $H_o$ ). Polymorphism statistics were performed on 25 individual females collected in the same OSR field in Ille & Vilaine, France.

Locus	Primer sequences (5'-3')	Repeat motif	Size (pb)	Size range	$N_a$	$H_e$	$H_o$	Null Alleles
<i>Th-ACRH 8</i>	F: CATAATTGTCGGCAGAAACG R: ATCTTGTCATTGTCCGTCC	(AG)11	289	276-284	2	0.30	0.25	0.06
<i>Th-AOD8 1</i>	F: TTGGTGTGGGATGATATCG R: TCAAAGGTCCTAAGTTCGTC	(CT)9	235	255-283	2	0.24	0.27	0.00
<i>Th-AXWO A</i>	F: GCTTCCATTACAAAGTTTACG R: AGTGCTGAAGTTTATTTCCC	(AG)11	157	151-165	2	0.53	0.50	0.00
<i>Th-B2M3 A</i>	F: ACACTAGAATAATTGCACGC R: GTAGCTGAATGACGACAAAC	(AG)10	239	235-237	2	0.50	0.63	0.01
<i>Th-BBCF N</i>	F: CTGACGCACATTCGTAAGG R: CAGTGATGTTAACC GA ACTTGGC	(AG)9	189	187-191	2	0.17	0.12	0.00
<i>Th-BXXG G</i>	F: GCCCGAATCTCATTAAACG R: GTTCACAGCCTTAATAAGAACC	(GA)9	344	338-248	3	0.33	0.38	0.00
<i>Th-C3H5 G</i>	F: ACCTTCATTCACTCTCCATC R: GTTGAGCTGATAATTGTGGC	(CT)9	244	227-239	3	0.77	0.73	0.00
<i>Th-C56F P</i>	F: GAAGACCGGAAACAGAGC R: CGGATTTTCGCTTCAGG	(CT)11	229	227-229	3	0.46	0.48	0.00
<i>Th-CIET 7</i>	F: TGACGTGGGAGAAACAGAG R: GCTTTGCCACCTCGTTC	(GA)10	163	161-165	3	0.29	0.33	0.02
<i>Th-cons4 7</i>	F: GAACATTGTTGGCTTATAACCC R: CATAGGGCAAAGACTAGCG	(CA)15	209	207-243	4	0.47	0.42	0.00
<i>Th-cons52 1</i>	F: TTCTATATTTGGGCTGTGC R: ACGTCATACAAGCACTATCC	(GA)13	331	319-329	4	0.48	0.50	0.03
<i>Th-Cons65 1</i>	F: TTCCCTTCGACGTGTCC R: ATTAGAGTTTACGACCATTGTG	(TC)10	289	283-285	4	0.50	0.44	0.00
<i>Th-CTUR T</i>	F: GTCTTTC CA ATTCTCTGCAC	(TG)9	150		4	0.20	0.15	0.07

	R: ACTATCTCGCCCATTTTCAC			149-141				
<i>Th-CWJY 8</i>	F: GCTGTTTTAAGTGCTTTGAAC R: GATGGCGAATCGTGCG	(CA)9	198	195-197	5	0.48	0.41	0.05
<i>Th-DAXQ K</i>	F: TTAGAATAGCGGTTTGAATC R: TGTATCATGGGAAGTACGTC	(CT)10	209	204-214	6	0.14	0.15	0.00
<i>Th-DB9S W</i>	F: CCCTTTGATCGTTAACTCCC R: CCTCGGTATTCCCCATTTAC	(CT)12	248	244-248	7	0.41	0.44	0.00

### 3.2. Genetic variability in *T. heterocerus* populations

The mean number of alleles ranged from 3.00 to 3.38 (mean = 3.27), the gene diversity ( $H_E$ ) ranged from 0.36 to 0.44 (mean = 0.40) and proportion of heterozygotes ( $H_O$ ) ranged from 0.32 to 0.41 (mean = 0.37) (Tableau 5.3). We estimated  $HWE$  and LD on the females of Austria, Estonia and France (Ile & Vilaine). The three populations did not deviated from  $HWE$  and no significant LD was detected for the 359 microsatellite pairs ( $p < 0.00014$ ).

Tableau 5.3 : Genetics characteristics of *T. heterocerus* in the five European populations (mean [SE]).  $A_r$ : Allelic richness.

	N males/females	$H_E$	$H_O$	N alleles	$A_r$
Austria	10/10	0.45 [±0.23]	0.38 [±0.24]	3.19	3,00 [± 1.54]
Estonia	12/10	0.36 [±0.21]	0.35 [±0.21]	2.88	2,68 [± 0.88]
France (Eure)	25/1	0.32 [±0.21]	0.33 [±0.21]	3.13	2,51 [± 1.10]
France (Ille & Vilaine)	0/25	0.39 [±0.15]	0.38 [±0.16]	3.19	2,65 [± 0.90]
Sweden	18/0	0.19 [±0.24]	-/	1.94	1,81 [± 0.99]

### 3.3. Population structure of *T. heterocerus*

The genic differentiation was significant for all the population pairs ( $p < 0.05$ ). The global  $F_{ST}$  was of 0.15 ( $p < 0.001$ ). Based on their microsatellite genotypes, the number of clusters,  $K$ , to which the 111 individuals (males and females) could be assigned was estimated using the software Structure assuming a prior on the location and for  $K$  between 1 and 10. The model that best described the genetic data was with  $K = 2$  (Figure 5.2).

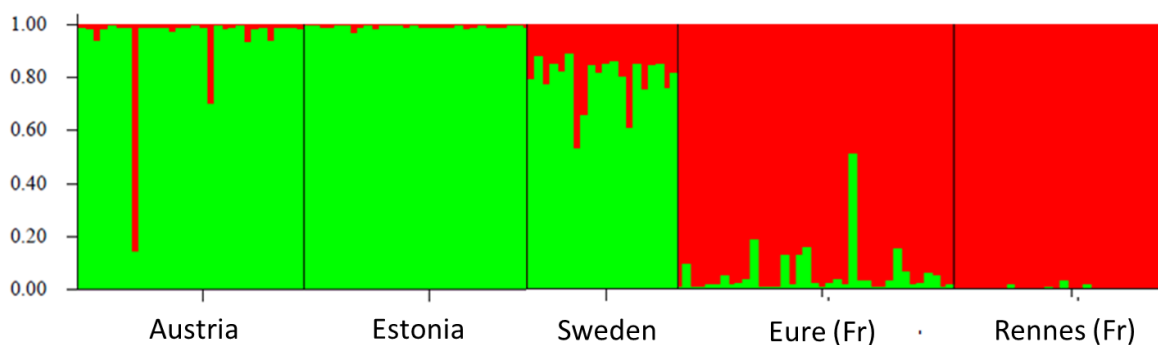


Figure 5.2: Bayesian assignment of *T. heterocerus* individuals from 5 populations to each of the  $K = 2$  identified clusters using *Structure* software on microsatellites.

### 3.4. Sibship assignments and distances

We performed 5 models of sibship assignments with the software COLONY, one for each of our population samples (Austria, Estonia, Sweden and France (Eure and Ille & Vilaine)). The number and percentage of full sibs was similar in all the populations (Tableau 5.4). The percentage of half sibs was also similar across populations, varying from 11 to 24 %. We also compared the number of full sibs and half sibs found in the adults from the Eure region ( $N = 128$ ) and in all the eggs ( $N = 93$ ). We found in this region 22 (0.27 % of the possible pairs) and 16 (0.37 %) full sibs and respectively 68 (0.83 %) and 90 (2.10 %) half sibs.

The effective population size  $N_e$  was similar among European populations except for the Austrian population which had a much higher  $N_e$  and Sweden with a lower  $N_e$  (Tableau 5.4).  $N_e$  calculated from all individuals collected in Eure was 268 with the adults and 35.2 [20, 62] with the eggs.

Tableau 5.4 : Mean number of half sibs (HSm maternal ship and HSp paternal ship) and Full sibs (FS) by population of *T. heterocerus*, and percentage within pairs of FS and HS possible with the by sites analysis.

Population	N	Possible dyads	FS nb	HSm nb	HSp nb	FS %	HSm %	HSp %	$N_e$
Austria	20	190	2	37	4	1.05%	19.47%	19.47%	13
Estonia	22	231	5	46	4	2.16%	19.91%	19.91%	13
France (Eure)	26	325	4	36	2	1.23%	11.08%	11.08%	23
France (Ille & Vilaine)	25	300	3	47	28	1.00%	15.67%	15.67%	12
Sweden	18	153	8	35	9	5.23%	22.88%	22.88%	9
All génération (Eure)									
France (Eure total)	128	8128	22	51	18	0.20%	0.60%	0.22%	268
France (Eure Eggs)	93	4278	16	45	33	0.37%	1.05%	0.01%	137

We also ran COLONY on the eggs considering the adults as putative parents. Out of the 92 eggs genotyped, seven were assigned to four different males among the 128 genotyped adults. These putative fathers were found at distances between 6,200 m and 16,700 m. This analysis showed also 6 pairs of full sibs: 3 from the same field and 3 in different fields. Two of these dyads were at 5,000 m apart, the last was à 12,000 m.

In our samples, seven cases of super-parasitism (two parasitoid eggs in the same larva) were detected. One of the pair was detected as full sibs.

#### 4. Discussion

We have developed 16 microsatellite markers on the Ichneumonid wasp, *Tersilochus heterocerus*. From a total of 5,368 microsatellite markers sequences, 40 were selected based on the number of repeated motifs and the size of amplified fragments. Less than a half of these selected sequences were kept as microsatellite markers as a lot of markers did not amplify or were monomorphic. We developed a consequent number of microsatellite loci compared to other ichneumonidae species. Indeed, in *Neotypus melanocephalus*, only nine polymorphic microsatellite were developed (Anton et al. 2006) but with a higher number of alleles (2 to 10). We tested if, even if the microsatellites markers were not very polymorphic, they were useful in population genetic analysis and sibship analysis.

The 111 adult parasitoids of the five European populations were assigned to 2 clusters *K*. The two populations from France were separated from populations from other countries in Europe. As our species is haplo-diploid, we used genic differentiation and it was significant for all the population pairs. This result was in agreement with the results of STRUCTURE but more precise as we differentiated all populations. The genetic structure of this species seems to be weak in France but stronger in Europe than its of his host, *B. aeneus* (Juhel et al., 2017b). The genetic diversity of the European populations was similar except for the Swedish population, in which it was lower without explanation.

We used sibship assignment analysis based on the microsatellite genetic markers we developed. Such sibship assignments depend on four assumptions (Jones and Wang, 2010): no deviation from the *HWE* in the populations, unlinked and selectively neutral loci, knowledge of population allele frequencies and no genotyping errors. Here, the *HWE* could be calculated only on populations with females, as these populations not deviating from the *HWE*, we considered that the other populations did not deviate either. Significant LD was not detected either in these populations. The other assumptions were assumed to be met.

The proportion of full sibs and half sibs was similar between European fields except for Swedish population. The proportion of half sibs was also low and similar between European populations. These results shows that the demographic patterns of this species are similar in Europe. As a result, the effective population size, *Ne*, was also significantly similar across populations, except for the Swedish population. As we had only males and so haploids, the diversity was lower and this could explain the low *Ne*.

We found couples of fathers and their sibs at long distances: 6 and 16 km. This distance seems to be high for a parasitoid. For example, the *Pleolophus basizonus* can travel 0.400 km in 2 days (Price, 1970). Parasitoids can use upwind anemotaxis for host-habitat location facilitating long distance dispersal (Williams et al., 2007). Other parasitoids of OSR are attracted by the oilseed rape volatiles (Jonsson & Anderson, 2008), we suppose it is the same for *T. heterocerus*. However, the distance at which parasitoids can detect these odors is still unclear. Nevertheless, there were some other OSR fields between the location of both individuals and that could be surprising that individuals do not stop in them. In any cases, as we detected only seven pairs of father-sibs, these distances have to be considered with caution and as bad assignment is possible. COLONY is made to detect sibs and group individuals and could detect false positives (Jones & Wang, 2010).

In this study, we extracted the DNA of adult parasitoids but also of eggs. The amplification of microsatellite markers was successful for 86 % of the adults but only for 49 % of the eggs. This result could be explained by a better conservation of DNA for adults than eggs and an easier extraction. The eggs were kept in the larvae of pollen beetles for three months in 90 % ethanol and then kept alone in 90 % ethanol for one month. Eventually, the eggs had to be extracted from the larva less time before the DNA extraction. Detection of DNA of parasites in their host is often used (Zhu & Williams, 2002) but the extraction of DNA from small individuals is rarer and harder.

Despite the low polymorphism of the 16 newly developed microsatellite loci, they were useful for measuring genetic variations within populations of *T. heterocerus*. These markers were also useful in the sibship assignment though a little weak. These polymorphic microsatellite markers could be used in future population genetics studies of *T. heterocerus* but for sibship assignments, adding more markers could be helpful to detect full sibs.

### **Acknowledgments**

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## Supplementary Material

Table S5.1: Polymorphic microsatellite loci selected in this study with reference to the labelled dye of the forward primer and the molar concentration (C) of each locus each multiplex. 6-FAM (6-carboxyfluorescein), HEX (hexachloro-fluoresceine), TAMRA (carboxy-tetramethyl-rhodamine), ATTO 565 (Rhodamine dyesclass).

Multiplex 1	Dye	C (nM)
<i>Th-B2M3A</i>	6-FAM	0.050
<i>Th-cons651</i>	6-FAM	0.100
<i>Th-CIET7</i>	Tamra	0.500
<i>Th-DAXQK</i>	Tamra	0.500
<i>Th-C56FP</i>	Hex	0.250
<i>Th-Cons521</i>	Hex	0.100
<i>Th-CTURT</i>	Hex	0.074
<i>Th-AOD81-R</i>	Atto-565	0.100
<i>Th-BXXGG</i>	Atto-565	0.200
Multiplex 2		
<i>Th-BBCFN</i>	6-FAM	0.074
<i>Th-ACRH8</i>	Tamra	0.400
<i>Th-C3H5G</i>	Hex	0.300
<i>Th-CWJY8</i>	Hex	0.150
<i>Th-AXWOA</i>	Atto-565	0.150
<i>Th-cons47</i>	Atto-565	0.250
<i>Th-DB9SW</i>	Atto-565	0.200

Table S5.2 : Characterization of 16 polymorphic microsatellite loci in 8 *Phradis interstitialis* samples collected in a seed in the Eure French department.. Sizes of the PCR products, number of alleles.

Loci	Alleles	Size range
<i>Th-ACRH8</i>	2	280-286
<i>Th-AOD81</i>	1	255
<i>Th-AXWOA</i>	2	157-165
<i>Th-B2M3A</i>	1	235
<i>Th-BBCFN</i>	1	187
<i>Th-BXXGG</i>	4	338-352
<i>Th-C3H5G</i>	2	227-237
<i>Th-C56FP</i>	2	225-227
<i>Th-CIET7</i>	2	163-165
<i>Th-cons47</i>	2	209-241
<i>Th-cons521</i>	1	317
<i>Th-Cons651</i>	2	281-283
<i>Th-CTURT</i>	1	147
<i>Th-CWJY8</i>	3	191-195
<i>Th-DAXQK</i>	1	208
<i>Th-DB9SW</i>	1	246





## Chapitre 6

L'impact des éléments du paysage sur  
l'abondance d'un parasitoïde est en partie  
lié à l'abondance de son hôte



## **Chapitre 6 : L'impact des éléments du paysage sur l'abondance d'un parasitoïde est en partie lié à l'abondance de son hôte**

La partie précédente montre que les parasitoïdes possèdent une structure génétique assez forte en Europe mais pas en France. Cette structuration est plus forte que celle trouvée chez les méligèthes suggérant des flux de gènes moins importants que pour les méligèthes. Dans ce chapitre nous cherchons à caractériser la présence des parasitoïdes dans les parcelles de colza à l'échelle locale. En particulier, nous nous sommes intéressés à l'effet de la présence de champs cultivés en colza l'année précédente pouvant révéler des corrélations géographiques révélatrices de capacités de dispersion limitée. Par ailleurs, le taux de parasitisme de cette espèce est plus élevé dans les paysages complexes, suggérant un impact direct des éléments semi-naturels sur le cycle de vie des parasitoïdes. Cependant, l'impact des paysages sur les parasitoïdes est généralement étudié via le taux de parasitisme, mais très rarement via le nombre de parasitoïdes adultes et leurs œufs. De plus, les études d'éco-physiologie des parasitoïdes mettent fréquemment en exergue l'importance de l'attraction des parasitoïdes par leur hôte (éventuellement via les odeurs de plantes attaquées) alors que les études basées sur les taux de parasitisme considèrent une simple relation linéaire entre présence de larve et présence d'œufs de parasitoïdes. Dans la mesure où les éléments semi-naturels sont très importants pour la biologie du ravageur, ils peuvent représenter un effet confondant lors de l'analyse des corrélations entre paysage et présence de parasitoïdes.

Nous nous sommes donc intéressés à la fois aux parasitoïdes adultes et au parasitisme en prenant explicitement en compte la présence de méligèthes comme un facteur explicatif. Nous avons échantillonné des parasitoïdes adultes, des méligèthes adultes et des larves, parasités ou non dans 96 parcelles de colza de l'Eure. Nous avons déterminé 4 variables paysagères: périmètre des forêts, périmètre du colza de l'année précédente, superficie du colza de l'année, superficie du colza de l'année précédente non labouré, chacune dans un rayon tampon de 0,3 km, 0,9 km, 1,5 km, 2,7 km. Nous avons estimé leur effet ajouté à l'abondance des larves et des adultes de méligèthes sur les parasitoïdes adultes et l'abondance des œufs et sur le taux de parasitisme à l'aide de modèles linéaires généralisés.

Ce travail fait l'objet d'un article qui sera prochainement soumis à une revue à comité de lecture et s'intitulera « **The impact of landscape elements on the abundance of a parasitoid is partly related to the abundance of its host.** ».





## **The impact of landscape elements on the abundance of a parasitoid is partly related to the abundance of its host.**

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### **Abstract**

A large portion of crop pests and their natural enemies are influenced by crop area but also non-crop areas. *Tersilochus heterocerus* Thomson, is one of the main parasitoid of pollen beetle *Meligethes aeneus*, a major pest of oilseed rape. The parasitism rate of this species is known to be more effective in complex landscapes, and that non-crop areas such as woodlands and grasslands improve the biological control of this species. In general, studies on impact of parasitoids on pests control study parasitism rate but not the number of adult parasitoids and their eggs. The effect of crops and non-crops area on adult parasitoids and their parasitism rate is still vague. We sampled adult parasitoids, pollen beetles adults and larvae, parasitized or not in 100 oilseed rape fields in the Eure French department. We determined 4 landscape variables: perimeter of woodlands, perimeter of oilseed rape of the previous year, area of oilseed rape of the year, area of oilseed rape of the previous year not plowed, each in buffer radius of 0.3 km, 0.9 km, 1.5 km, 2.7 km. We estimated their effect added to abundance of pollen beetles larvae and adults on adult parasitoids and eggs abundance and on parasitism rate with generalized linear models. Here, we show that the abundance of pollen beetle adults strongly explains the distribution of eggs and adult parasitoids. The perimeters of wood also have a positive effect in 0.9 km on adults but not on eggs. Others landscape elements did not impact parasitoids abundance. Parasitism rate was negatively impacted by the perimeters of woodlands and by the area of oilseed of the year. Our results suggest that the landscape is not the main element for parasitoids and they are influenced in a major part by pollen beetles.

Keywords: biocontrol, *Brassicogethes aeneus*, parasitoids, *Tersilochus heterocerus*, woodland

## 1. Introduction

Natural enemies are significant in the control of crop pests (Büchi et al., 2002). The majority of these species is dependent on non-crop areas as shelter, breeding or feeding sites (Bianchi et al., 2006). The intensification of agriculture is a major threat to natural enemies through the loss of hedgerows and grasslands (Bianchi et al., 2010), the use of pesticides and deep tillage (Bianchi et al., 2010). These negative effects of intensification of agriculture are particularly visible on parasitoid wasps, one of the main biological control agents in agroecosystems (Hawkins et al. 1997), often dependent on non-crop areas (Thies et al., 2005) and on agricultural soils (Nilsson, 2010).

Oilseed rape (*Brassica Napus L.*) (OSR), is an economically significant crop in Europe, attacked by many of pest species such as the pollen beetles, *Brassicogethes aeneus* (formerly named *Meligethes aeneus*). This major pest of OSR damages the flowers by puncturing and ovipositing in buds (Williams, 2010). *Tersilochos heterocerus*, is the main parasitoid of this pest in Western Europe (Ulber et al., 2010). It lays his eggs into pollen beetle larvae while they are in the flowers. After the pollen beetle larvae drop and pupate into the soil, the parasitoid egg develops to fully replace the pollen beetle larvae in the pupa staying in the soil and overwintering in it until the following spring (Williams, 2006). Like other parasitoid wasp, this species is supposed to be impacted by the landscape structure, as the parasitism rate is higher in complex landscapes (Thies & Tschardt, 1999), in particular those rich in woodlands and grasslands (Rusch et al., 2012a). The difficulty for parasitoid wasps to disperse in high open field spaces (Thies et al., 2008) and their dependence on pollen and have been cited to explain this relationship (Rusch et al., 2013). The woodlands and grasslands have also a positive effect on pollen beetles and their effect on *T. heterocerus* could be an indirect effect of their effect on this host. Moreover, all these studies were focused on parasitism rate and not on the adult parasitoids and their eggs.

*T. heterocerus* is known to be negatively impacted by deep tillage (Williams, 2006). The soil tillage following the harvest of the oilseed rape crop (OSR) could reduce their survival as it could bury the pupas or expose them at the surface of the soil (Nilsson, 2003, Klingenberg & Ulber, 1994). Nevertheless, the effect of different tillage regimes has not always been found significant (Hanson et al., 2015). Crop rotation variations and environmental changes might have seem to have more impact on their abundance (Thies et al., 2008) and highlight the need of increase the knowledge on these species to enhance their biological control.

Here we investigate how both adult parasitoids and eggs abundance relate to the landscape and to pollen beetle abundance in a joint capture of adult parasitoids, adult pollen beetles and larvae (parasitized or not) in 96 fields in the North-West of France.

## 2. Materiel and Methods

### 2.1. Study site

The study site was an area of 12 km by 20 km centered on 48°56'01.6"N 1°21'15.8"E situated in the Eure departement in North-Western France, (Figure 6.1). This area is composed of plateau with few hedgerows, grasslands and woodlands cut by a valley with plenty of those.

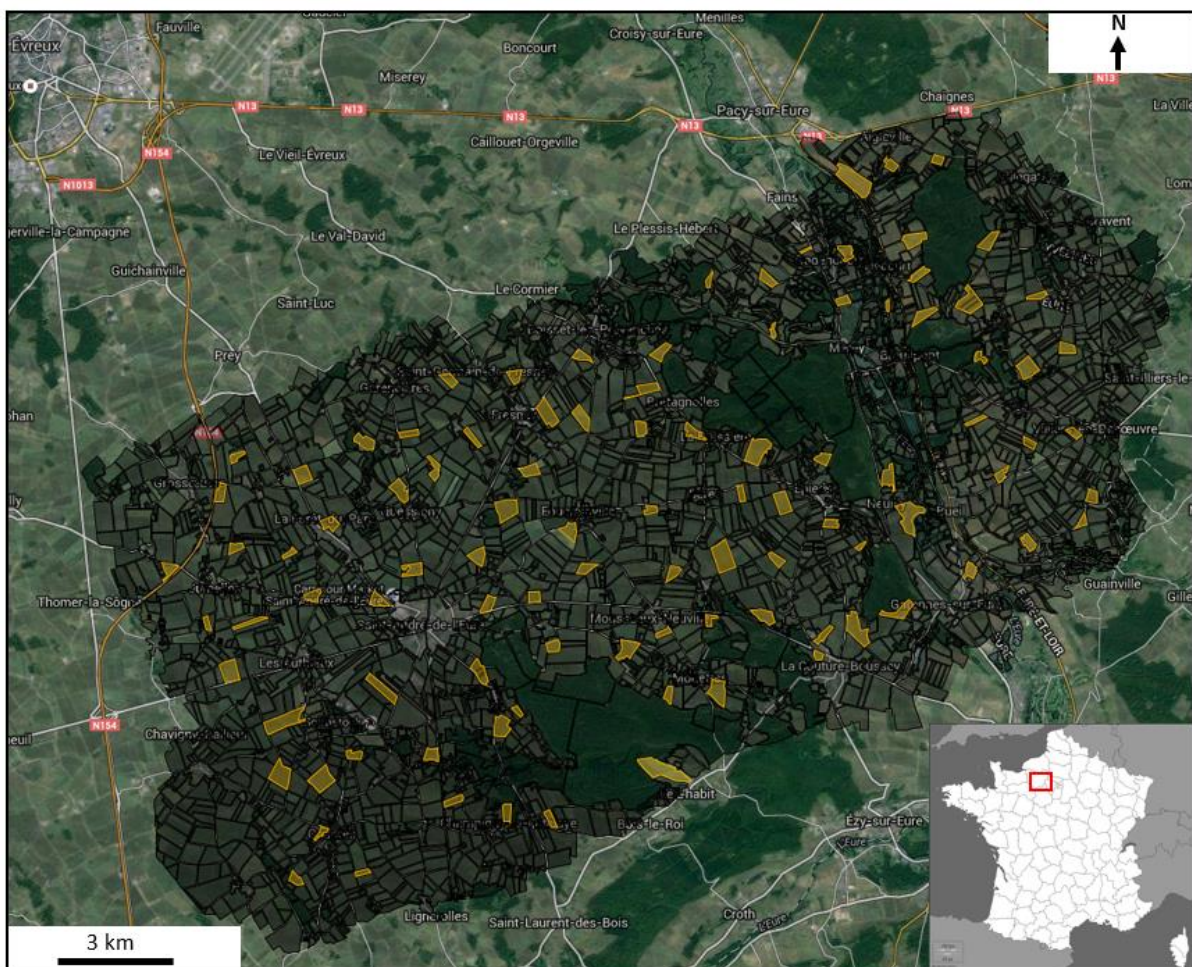


Figure 6.1 : Study site in the Normandy region in 2016 with sampled fields.

### 2.2. Mapping and geomatics

We mapped forests and field delimitations of the study site using aerial photographs (GoogleMyMaps pixel size: 0.5 m, ©2017 TerraMetrics). We also mapped the crops of the year

and the year before based on field surveys of the area. We also mapped tillage regimes by seeing the residuals of the previous crop and the traces of the tillage. Hedgerows were not mapped as they were more difficult to identify from aerial photographs. We calculated woodland areas and distances of each sampling point to woodlands using the R package *rgeos* (Bivand & Rundel, 2013) using the Universal Transverse Mercator (UTM) projection. All geomatics treatment and statistical analyses were performed using R software (R Core Team 2017).

### ***2.3. Insect sampling***

We selected 100 OSR fields distributed to cover all the area (Figure 1). On May 18<sup>th</sup> 2016, in each of these fields, we sampled by beating 5 OSR flowers stems in plastic bags at 15 points separated by 2 m. At this date, this method allowed us to sample jointly adults and larvae of pollen beetles, adult parasitoids and parasitized larvae of pollen beetles. Adult parasitoids were identified by their wing and antennae according to Osborn (1960). The eggs of *T. heterocerus* were seen in larvae of pollen beetles by transparency under a binocular magnifying glass. Among the 5151 adult pollen beetles captured, 1000 individuals equally distributed among the fields were identified by comparing their meta-femur (Audisio, 1993). Most pollen beetle were identified as *B. aeneus* (99%).

### ***2.4. Statistical analysis***

We performed generalized linear models (glm) to determine the effect of landscape elements on adult parasitoid abundance, on parasitoid egg abundance and parasitism rate. We modelled the parasitism rate with a binomial distribution. The parasitism rate is usually used in studies on parasitoids. Here we compared this model to models using the eggs of this parasitoids and the adults. We used a negative-binomial distribution with a log-link function to account for the overdispersion in adult *T. heterocerus* and *T. heterocerus* eggs. We used as explicative variables landscape variables known to be correlated with parasitoid abundance: the proximity of OSR fields the previous year (from which they emerge), the proportion of OSR of the year (where they reproduce) and the proportion of woodlands verges and grasslands that could be nectar resources. We took only the woodland verge as we thought they could be sources of nectar for parasitoids. As the plow is known to reduce parasitoids abundance (Hanson et al., 2015), we added the proportion of plowed OSR fields of the previous year. The proportion of these elements were assessed in circular buffers of radius of 0.3, 0.9, 1.5 and 2.7 km around the sampled fields as adult parasitoids are supposed to disperse at 1 km (Rusch et al., 2012a). We used in the regression the normalized log of all land use explicative variables: log to keep a

proportional relationship between abundance and surfaces and normalized to allow comparison of effect sizes between the variables. We added to the models of abundance the normalized log abundance of adult pollen beetles and larvae. For the parasitism rate, we only added the adult pollen beetles as larvae were already accounted for through the rate. For each of the three models (adults, eggs, and parasitism rate), we selected the best buffer radius comparing their AIC (Akaike, 1987). We did not include proportion of grasslands as they were too correlated to proportion of OSR of the year and of the previous year (0.84 and 0.70 respectively).

### 3. Results

Among the 162 adult parasitoids caught, 138 (mean per 60 plants 1.51 with 95 % quantiles [0.0, 11.8]), were *T. heterocerus*, 19 *Phradis interstitialis* and 5 other undetermined species. Only 10 of the 138 *T. heterocerus* were females. We caught a total of 5151 pollen beetles adults (mean = 46.82 [0.73, 174.78]), 7912 pollen beetles larvae (mean per 60 plants = 71.93 [0.00, 530.05]) and a total of 340 parasitized pollen beetles larvae (mean per 60 plants = 3.09 [0, 17]). The mean (per 60 plants) parasitism rate by field was 5.14 % ( $\pm$  0.09 %).

First, we performed a glm explaining the parasitism rate of pollen beetles by *T. heterocerus*, by landscape elements. The buffer radius of 2.7 km was selected for the parasitism rate model (519.1, 20 points better than the second model, the model with buffer radius of 1.5 km, Tableau 6. 1), explaining 10.57 % of the variance. The parasitism rate was negatively impacted by the perimeter of woodlands and the perimeter of OSR of the previous year.

Tableau 6. 1 : Results of the generalized linear model of the parasitism rate of pollen beetles (PB).

Variable	Estimate	Std. Error	Pr(> z )
Abundance adults PB	0.116	0.06	0.054 .
Perimeter of woodlands (2.7 km)	-0.167	0.07	0.013 *
Perimeter of previous OSR (2.7 km)	-0.087	0.06	0.150
Proportion of OSR of the year (2.7 km)	-0.227	0.08	0.007 **
Proportion of OSR of the previous year not plowed (2.7 km)	-0.069	0.07	0.068 .

Second, we performed a model describing the abundance of parasitoid eggs (Tableau 6.2). We selected the best buffer radius of the landscape variables by comparing the AIC of the models. The selected model was the model with a buffers radius of 2.7 km, explaining 48.5 % of the variance (AIC of 404.29, 4 points better than the second model with a buffer radius of 1.5 km). Only pollen beetle larvae abundance had a significant effect on the abundance of parasitoids

eggs. This suggest that the landscape variables had not a significant effect. Moreover, this model had a better AIC than the model describing the parasitism rate.

Tableau 6.2 : Results of the generalized linear model of the abundance of pollen beetles larvae parasitized explained by landscape variables. PB: pollen beetles

Variable	Estimate	Std. Error	Pr(> z )
Abundance adults PB	0.09	0.13	0.495
Abundance larvae PB	0.98	0.14	5.59E-12 ***
Perimeter of woodlands (2.7 km)	-0.11	0.13	0.375
perimeter of previous OSR (2.7 km)	-0.16	0.12	0.195
Proportion of OSR of the year (2.7 km)	-0.22	0.16	0.163
Proportion of OSR of the previous year not plowed (2.7 km)	-0.02	0.12	0.867

Finally, the adult parasitoids were used as normally they were less constrained by the abundance of pollen beetles. We performed a GLM to determine the effect of the landscape variables and of pollen beetles abundance on parasitoid adult abundance (Tableau 6.3). The model with buffers of 0.9 km was which with the best AIC (AIC of 251.44, better than the second model, with the buffer radius of 1.5 km, by 3 points) and explained 44.34 % of the variance. Pollen beetle adults and larvae were positively and significantly correlated with adult parasitoid abundance. Among the 4 landscape variables, only woodlands perimeter had a positive and significant impact.

Tableau 6.3 : Results of the generalized linear model of the abundance of adult parasitoids given landscape variables and pollen beetle abundance.

	Estimate	Std. Error	Pr(> z )
Adult pollen beetle abundance	0.899	0.252	0.0004 ***
Pollen beetle larva Abundance	0.534	0.229	0.0195 *
Perimeter of woodlands (0.9 km)	0.799	0.227	0.0004 ***
Perimeter of previous OSR (0.9 km)	0.381	0.215	0.0761 .
Proportion of OSR of the year (0.9 km)	-0.307	0.239	0.1980
Proportion of OSR of the previous year not plowed (0.9 km)	-0.008	0.161	0.9630

#### 4. Discussion

In this study we showed that the parasitism rate of pollen beetles by *T. heterocerus* was negatively correlated to woodland verges and OSR fields of the year. We compared it to a model accounting only on eggs and not on a rate to have a linear relation and not a proportional relation. With this second model, only the abundance of pollen beetles larva was significant, suggesting a weak effect of landscape on these abundance. To verify it, we performed the same model with adult parasitoids, and showed a positive effect of the adults and larvae pollen beetles

and of the perimeter of woodlands. This change in the sign of the effect of woodlands shows its weak effect on parasitoids abundance.

We performed a model explicating the parasitism rate by landscape variables and pollen beetles larvae abundance. This models showed a negative effect of OSR fields of the year. This result is consistent with previous studies showing a dilution effect of OSR in the same region (Rusch et al., 2011) whereas other studies in other countries found parasitism rate was unaffected by OSR area (Zaller et al., 2009, Thies et al., 2003). These divergences could be explained by differences in the landscape structure and arrangement of landscape elements in these regions. Nevertheless, we found a negative effect of woodland perimeters whereas Rusch et al (2011) found a positive effect of the proportion of woodlands. This could suggest parasitoids prefer dense woodlands, which could protect them as a barrier from wind.

This model was weaker than model explaining abundance of eggs. The model with eggs was more linear than the model with parasitism rates and could better explain what can influence them. In this model only the abundance of larvae of pollen beetles was significant. Finally, we compared it to the same model but explaining abundance of adult parasitoids. The abundance of both larvae and adults pollen beetles were positively significant.

Only one landscape elements had an impact on adult parasitoids, the perimeter of woodlands, and none had an effect on parasitoids eggs. The positive effect of this element could be explained by the source of flowers present in the verges of woodlands and could be a relay for parasitoids. Woodlands are a key element for overwinter of pollen beetles (Rusch et al., 2012b) and this behavior of pollen beetles is likely an indirect effect of woodlands on adult parasitoids. Moreover, this effect have to be accounted with caution as its sign was variable between models.

Once accounted for adult pollen beetles abundance, others landscape elements as proportion of oilseed rape of the year and of the previous year had no effect on adult parasitoids and eggs abundance. These elements had an effect on *T. heterocerus* in other studies (Rusch et al., 2011). Here, unlike in previous studies, we added adult pollen beetles abundance in our models and it was became the main effect, offsetting most of the correlation with landscape elements.

We showed that the variability of abundance both for adult parasitoids and their eggs was mainly correlated with pollen beetle abundance, whether adults or larvae. Natural enemies of pest crops are often attracted by volatile organic compounds (VOCs) emitted by crops when they are damaged by their pests (Ahuja et al., 2010). Oilseed rape also emits VOCs when it is damaged (Ahuja et al., 2010), including when damaged by pollen beetles (Lindkvist, 2003). *T.*



*heterocerus* is known to be attracted by this odor (Lindkvist, 2003, Jonsson et al., 2005). Moreover, the odor of the pollen beetle larvae is more attractive than uninfected OSR plant (Berger et al., 2015), this could explain the positive effect of pollen beetles larvae on adult parasitoids in our results. The attractiveness of pollen beetles adults could be due to the VOC emitted when they eat OSR flowers coupled with the fact that if there are pollen beetles adults, there are probably larvae. *T. heterocerus* is also attracted by yellow flowers (Berget et al., 2015) as pollen beetles, this could also explained an indirect effect of the attractiveness of pollen beetles by parasitoids. The effect of pollen beetles larvae on parasitoids eggs could also be explained by the attractiveness of adult parasitoids by pollen beetles larvae (Berger et al., 2015).

Though *T. heterocerus* overwinters in soils of OSR fields, the effect of tillage methods was unclear. Some studies showed that the parasitism rate by *T. heterocerus* was negatively related to the ploughed oilseed rape fields around the sampling point (Rusch et al., 2012a) whereas Hanson et al., (2015) found no effect of tillage method. Here, we did not found effect of the type of tillage, confirming the ambiguity of this effect.

The buffer radii of 2.7 km was selected for the parasitism rates, and was the only one we can measure as for other models, pollen beetles obfuscated the effect of landscape elements. It is larger than buffer radius found in another study in the same region, showing an effect of landscape in buffer radius of 1.5 to 2 km (Rusch et al., 2011). As the pollen beetles abundance decreased between both studies, parasitoids could have to travel farthest for finding pollen beetles.

The parasitism rate by *T. heterocerus* was low, with a mean of 5 % per field, to be compared in the same region to 30 % on average and a maximum of 80 % ten years before (Rusch et al., 2011). Nevertheless in other regions, parasitism rates as low as 1 % have been reported (Zaller et al., 2009). The large majority of *T. heterocerus* (85 % vs. 12% *Phradis interstitialis*) is consistent with previous report in the same region (*T. heterocerus* 95 % vs. 4 % *Phradis interstitialis* and *Phradis morionellus*, Rusch et al., 2011). These three species are known to be the most abundant and common in Europe (Nilsson et al 2003, Jonsson et al., 2004).

We caught a majority of males for both species (93 % for *T. heterocerus* and 79 % for *P. interstitialis*). This predominance of males could be an effect of the date of sampling, as *T. heterocerus* males seems to emergence two weeks earlier than females (Jourdheuil, 1960). It could also be due to the behavior of males, they tend to be aggregated (Jourdheuil 1960) and we found females alone.

Moreover, we found an effect of landscape variables at a buffer radius of 2.7 km, it was the higher buffer radii we can use as we did not have the land use at more than 3 km around the border fields. Nevertheless, at our knowledge it was the higher buffer radii tested for landscape variables for this species. Further analysis could explore these effect at larger scales. Moreover, this study was conducted on only one year, to account for effects of the weather, a second or third year could adjust our results.

This study suggests that the higher rates of parasitism of the pollen beetle by the parasitoids in complex landscapes might less due to the landscape itself than to an attraction of the parasitoids more than proportionally with the abundance of pollen beetle. A very strict correspondence between the needs of the pollen beetle and of its parasitoid might also explain the lack of influence of the landscape on the parasitoids. In any case, our results suggest a very strong capacity of the parasitoid to disperse to pollen beetle infested sites as this completely erases the spatial correlation with their emergence sites; other relevant spatial relationship with other landscape elements might be equally weakened. Studying more precisely the dispersal of *T. heterocerus*, its determinants and limits might be key to best favor this natural enemy.

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# Chapitre 7

Discussion générale



## Chapitre 7 Discussion générale et perspectives

La première partie de cette discussion présente les faits marquants de ce travail. La seconde met en relation les résultats entre eux et avec la littérature scientifique existante. Les limites de ce travail sont discutées dans une troisième partie. Dans une quatrième partie, nous montrons l'intérêt appliqué de nos résultats pour la gestion des méligèthes et pour améliorer la régulation apportée par les parasitoïdes. Enfin dans une dernière partie, les perspectives de ce travail sont développées.

### 1. Les faits marquants de ce travail

Au cours de ce travail, des études ont été menées à différentes échelles spatiales et à différents moments du cycle de vie des méligèthes *Brassicogethes aeneus*, et de leur parasitoïde principal, *Tersilochus heterocerus*. Pour cela, nous avons mobilisé deux approches : l'une basée sur la dispersion de marqueurs génétiques, en utilisant des marqueurs spécifiques, ici des microsatellites et l'autre basée sur la dispersion des individus, en les piégeant dans leurs habitats respectifs. Pour cela, nous avons développé des marqueurs microsatellites pour les méligèthes, *Brassicogethes aeneus* et leur parasitoïde principal *Tersilochus heterocerus*, ce qui n'avait encore jamais été réalisé. Cette avancée technique a permis de mesurer la différenciation génétique entre populations en Europe et l'apparentement entre individus collectés dans une même parcelle ou dans des habitats proches. Les mesures d'apparentement ont été utilisées pour estimer la taille efficace des populations. Les analyses génétiques montrent peu de structuration des populations de méligèthes, peu d'apparentement même à faible échelle et elles ont permis de pointer l'importance de la taille de la population. Elles ont aussi montré une structure génétique plus faible des populations de parasitoïdes que celles de leur hôte.

Les captures d'individus dans le paysage dans des zones plus restreintes ont par ailleurs permis de progresser sur la biologie du méligèthe: la distance potentielle qu'il peut parcourir pendant la phase de dispersion printanière, le rôle de certains habitats pendant le printemps et l'été et tout particulièrement le rôle des fleurs dans ces habitats ont été estimés. Nos résultats ont montré que les méligèthes utilisent de nombreux habitats, ce qui complique leur gestion. En effet, les habitats impliqués sont aussi ceux utiles aux pollinisateurs et à leurs ennemis naturels. Nous avons aussi progressé sur la biologie du principal parasitoïde : le rôle indirect du paysage



semble être principalement lié à ses effets sur les méligèthes. Seuls les bois ont dans nos données un effet direct sur cette espèce.

## **2. Retour sur les résultats et enseignements sur l'écologie et la biologie de ces organismes**

### **2.1. La structure génétique des méligèthes et de leur parasitoïde à l'échelle européenne**

A l'aide de 12 marqueurs microsatellites développés dans ce travail, nous avons étudié la diversité et la structure génétique de 18 populations de méligèthes à des échelles spatiales emboîtées : dans différents pays européens, dans différentes régions de France et dans différentes parcelles de l'Eure. Parallèlement, nous avons développé 16 marqueurs microsatellites pour étudier la structure génétique de 5 populations européennes de parasitoïdes.

Le polymorphisme des marqueurs microsatellite des méligèthes est en moyenne de 6 allèles par marqueurs, tandis que celui des parasitoïdes n'est que de 3 allèles par marqueurs. La probabilité que deux méligèthes d'une même population aient par hasard le même génotype aux 12 locus développés est inférieure à  $9 \times 10^{-5}$ . La probabilité que deux *T. heterocerus* d'une même population aient par hasard le même génotype au 16 locus développés est sensiblement plus élevée :  $1,6 \times 10^{-3}$ .

Nos résultats sur la structure génétique des populations de méligèthes sont en accord avec d'autres études menées avec des marqueurs différents qui ont montré une faible structuration génétique à l'échelle du continent européen (Ouvrard et al., 2016 ; Kazachkova et al., 2007). Seules les populations de méligèthes les plus éloignées en Europe étaient différenciées génétiquement. Aucune structuration génétique est observée en France, ce qui confirme l'absence de structuration relevée précédemment en Suède (Kazachkova et al., 2007) et en Grande-Bretagne (Ouvrard et al., 2016).

Les analyses de la diversité génétique dans les populations suggèrent que 7 des 18 populations européennes analysées ne seraient pas à l'équilibre mutation-dérive ce qui pourrait refléter une expansion des populations de méligèthes liée à l'expansion de la culture du colza en Europe. Cette hypothèse est aussi avancée par Hokkanen (2000) qui a montré que les méligèthes s'adaptent en augmentant leur production d'œufs avec l'augmentation de la quantité de colza disponible. Beaucoup de brassicacées sauvages existent, et auraient été les hôtes des méligèthes avant la culture du colza. En augmentant les surfaces cultivées en colza on a ainsi augmenté les hôtes potentiels des méligèthes. Ce phénomène n'est probablement pas limité aux méligèthes mais devrait aussi affecter d'autres ravageurs de grandes cultures (Altieri & Nicholls, 2004).

L'augmentation des pratiques en monoculture y a grandement contribué en réduisant la biodiversité et en homogénéisant les écosystèmes (Tilman, 1999).

Chez les parasitoïdes, la différenciation génétique des populations est significative à l'échelle de l'Europe mais pas à l'échelle de la France, tout comme pour les méligèthes. Cependant, la structuration génétique des populations de *T. heterocerus* semble être plus forte que celle de son hôte, les méligèthes. Ce résultat est aussi visible pour d'autres espèces. C'est notamment le cas de *Neotypus melanocephalus*, un parasitoïde hyménoptère du papillon *Maculinea nausithous* (Anton et al., 2007). Ces différences de structuration génétique pourraient être dues en partie à des tailles de populations plus faibles chez les parasitoïdes par rapport à leurs hôtes.

Ces résultats tendent donc à suggérer deux éléments importants pour ce ravageur : que les populations de méligèthes sont grandes et qu'ils se déplacent probablement à de très larges échelles spatiales. Compte tenu des autres résultats acquis dans le chapitre 3 sur la phase de dispersion printanière, cette capacité de progression forte est peut être liée à la phase de dispersion estivale. Pendant l'été les organismes cherchent à se nourrir dans des conditions plus favorables et pourraient ainsi se déplacer sur de plus grandes distances que les 2 premiers kilomètres observés au printemps. Ce résultat est corroboré aussi par le fait que les résistances aux insecticides détectées il y a 20 ans en France et il y a 17 en Suède (Hansen et al., 2003) se sont propagées dans beaucoup de pays d'Europe rapidement.

### ***2.2. Hétérogénéités au sein d'un paysage et rôle des différents habitats***

Nous nous sommes ensuite intéressés au fonctionnement des populations de méligèthes et de parasitoïdes en fonction de l'hétérogénéité du paysage dans le département de l'Eure.

Nous avons échantillonné des méligèthes dans des parcelles de colza deux années consécutives aux mêmes dates, entre mi-mars et fin avril. Les deux années, le nombre de méligèthes par parcelle a atteint un pic début avril avec une moyenne de 6 méligèthes par pied de colza. Ce nombre est très variable entre les parcelles et en fonction du paysage dans lequel elles se trouvaient. Ainsi, dans un paysage avec peu d'habitats semi-naturels, nous avons trouvé moins d'un méligèthe en moyenne par pied de colza, tandis que dans les paysages complexes, nous avons observé jusqu'à 10 méligèthes par pied. Cette variabilité et cet effet du paysage ont déjà été mis en évidence par de nombreuses études (Rusch et al., 2013).

Nous avons également échantillonné en mai 2016 simultanément des larves et des adultes de méligèthes mais aussi des parasitoïdes adultes et des larves de méligèthes parasitées par des œufs de parasitoïdes. Là encore les abondances étaient plus faibles dans les zones simplifiées

et plus importantes dans les zones complexes. Ce résultat est en accord avec les précédentes études sur le sujet qui ont montré que le taux de parasitisme était plus élevé dans des paysages complexes (Thies & Tschardtke, 1999) avec de fortes proportions de bois et de prairies (Rusch et al., 2012).

Nous nous sommes demandés si le paysage affectait directement les abondances de parasitoïdes ou si son effet était indirectement lié à son effet sur les abondances de méligèthes. Nous avons démontré que ce qui comptait vraiment pour les parasitoïdes était la présence de méligèthes et dans une moindre mesure, la surface de lisière de bois. Ainsi la présence de prairies, n'a pas d'effet significatif sur les abondances de parasitoïdes quand les abondances de méligèthes sont prises en compte. Il en va de même quand on cherche à expliquer le taux de parasitisme et les abondances d'œufs de parasitoïdes. L'effet des prairies sur les parasitoïdes semble surtout un effet indirect de leur effet sur les méligèthes. De plus, nous n'avons jamais récolté de parasitoïdes dans des prairies malgré nos efforts répétés : différentes techniques (pièges jaunes ; filets fauchoirs ; collectes hebdomadaires d'avril à mai) présentées dans le matériel et méthodes global. Par contre, des parasitoïdes ont été trouvés dans les parcelles de colza dans les cuvettes jaunes et lors de quelques collectes au filet fauchoir. L'absence d'observation de parasitoïdes dans les prairies suggère que les parasitoïdes n'utilisent pas ou peu ces habitats, confirmant que les corrélations observées préalablement à notre travail entre prairies et présence de parasitoïdes sont dues à des confusions d'effets. Leur besoin de nectar est assuré par les fleurs de colza, ce qui corrobore les résultats de Rusch et al., (2013), qui montrent la présence de sucre principalement dans les parasitoïdes trouvés sur les parcelles de colza.

En parallèle, nous nous sommes demandé si ces prairies, mais aussi d'autres habitats semi-naturels tels que les jachères ou les bords de bois ou d'autres parcelles avaient une importance pour les méligèthes au cours de leur cycle. Nous avons mis en évidence que les méligèthes, après être sortis d'hivernation étaient fortement attirés par les fleurs jaunes ayant beaucoup de pollen, tels que les pissenlits présents dans les prairies, les bords de route ou les lisières de bois. L'attraction des méligèthes par les pissenlits avait déjà été mentionnée (Taimr et al, 1967). L'abondance de méligèthes diminuant progressivement dans ces habitats de mars à avril, nous supposons qu'ils peuvent être des relais pour les méligèthes en attendant que le colza fleurisse. En ce sens, leur effet pourrait être à double tranchant, d'une part ils peuvent favoriser le génotype de méligèthes à émergence précoce, particulièrement dommageables, d'autre part ils pourraient détourner les méligèthes des colzas non encore fleuri limitant ainsi leur nuisibilité.

En été, lorsque les méligèthes émergent des parcelles de colza, il reste peu de fleurs de colza fleuries. Nous avons trouvé un nombre important de méligèthes dans les prairies mais aussi dans les adventices des cultures. En effet, à cette période les bords de route et les prairies sont fauchés et sont donc peu fleuris. Les fleurs dans lesquelles nous avons trouvé des méligèthes avaient des caractéristiques de couleurs, de formes, de quantités de pollen et de nectar très variées en comparaison avec les fleurs observées au printemps. Nous avons trouvé beaucoup de méligèthes dans des coquelicots et des églantiers. Deux autres études avaient étudié les fleurs dans lesquelles pouvaient être trouvés les méligèthes lors de cette période et avaient trouvé des méligèthes dans un grand nombre de variétés de fleurs différentes (Free & Williams, 1978 ; Charpentier, 1985 ; Ouvrard et al., 2016), entre autre dans des fleurs similaires aux nôtres.

Ces résultats montrent que les méligèthes utilisent les fleurs présentes dans les habitats semi-naturels lors de leurs phases de dispersion mais aussi les adventices présentes dans les cultures. De plus il a été montré que les méligèthes utilisaient aussi les plantes ornementales en tant que ressources (Charpentier, 1985 ; Ahmed et al., 2013), et nous le confirmons avec certaines plantes ornementales trouvées dans l'environnement agricole sur lesquelles nous avons trouvé des méligèthes, telles que les mahonias et les pavots de Californie.

Les habitats utilisés par les méligèthes pour hiverner doivent encore être précisés. Dans une seule tente à émergence, dans les parcelles de colza, nous avons trouvé en moyenne 65 méligèthes adultes en juin 2015. Au printemps suivant, dans une seule tente de la même taille, mais placée dans les bois, nous avons trouvé en moyenne 3 méligèthes adultes. Ensuite, en mars, dans les parcelles de colza, nous n'avons pas placé de tentes mais échantillonné par frappage (en tapant du colza dans des sacs) des méligèthes et sur 10 plants nous avons collecté en moyenne 41 méligèthes adultes sur environ la même surface échantillonnée que pour les tentes. Le nombre de méligèthes échantillonnés dans les bois est donc très bas comparé à ce qui est échantillonné dans les colzas. Une explication pourrait être que le pourcentage de surface de bois est plus grand que le pourcentage de surface cultivée en colza mais il est respectivement de 22 % et 10 % ce qui n'est pas une différence assez grande comparée à la différence d'abondance. Une autre explication serait que les méligèthes s'agrègent dans les bois. Ils ont déjà tendance à le faire dans les parcelles de colza (Ferguson et al., 2003). De plus certains insectes ont tendance à s'agréger en hiver pour se protéger (Vulinec, 1990), les méligèthes pourraient donc avoir ce comportement, cependant, nous n'avons jamais observé de fortes abondances et au contraire beaucoup de tentes avec quelques méligèthes. Une autre hypothèse serait que les bois ne seraient pas le seul lieu d'hivernation des méligèthes. En effet, les

méligèthes hivernent dans la litière de feuilles (Rusch et al., 2011b), ce qui sous-entend dans les bois. Mais ils pourraient aussi hiverner dans la litière des haies, ce qui expliquerait qu'on ne trouve pas une grande proportion de méligèthes dans les bois. De plus, une part non négligeable de méligèthes émerge des prairies (Rusch et al., 2011b). Ce phénomène pourrait aussi expliquer que les bois ne sont pas un élément significatif dans les études se basant en Angleterre (Skellern et al., 2017). En effet ce pays possède peu de bois mais beaucoup de prairies et de haies.

### ***2.3. Dynamique des populations des deux espèces à une échelle régionale***

#### *2.3.1. Tailles efficaces des populations des deux espèces*

La taille efficace des populations a été mesurée à plusieurs moments du cycle de vie des méligèthes et entre deux générations pour les parasitoïdes. Aux différents moments du cycle de vie des méligèthes, nous avons trouvé peu de parentés, ce qui peut s'expliquer par la forte taille de population des méligèthes montrée dans le chapitre 1. De plus, la taille efficace des populations de méligèthes entre différents points d'une même parcelle étaient similaires. Ce résultat montre que les adultes se répartissent au sein d'une parcelle et qu'ils ont des pontes dispersées (Hopkins & Ekbohm, 1999). De plus, les tailles efficaces entre parcelle étaient similaires mais légèrement plus faibles que dans des parcelles éloignées des bois ce qui pourrait être dû à un moins grand nombre d'individus ayant accédé à ces parcelles. De même, les tailles efficaces étaient similaires à celles trouvées dans les prairies, mais légèrement plus faibles que dans les parcelles de colza ce qui n'était pas attendu. Ceci pourrait être lié à des individus capturés dans les prairies alors qu'ils venaient d'émerger des parcelles de colza et seraient la nouvelle génération de méligèthes.

Pour les populations de parasitoïdes, comme pour celles de méligèthes, nous avons estimé des tailles efficaces de même grandeur dans différentes parcelles échantillonnées. De plus, des effectifs efficaces équivalents ont été estimés sur deux générations consécutives de parasitoïdes.

#### *2.3.2. La dispersion des méligèthes*

La dispersion des méligèthes reste une question majeure pour mieux gérer cette espèce et en prenant notamment en compte le rôle du paysage dans ces déplacements à l'échelle d'une petite région. Nous avons utilisé différentes méthodes pour évaluer cette question, des modèles d'abondance aux modèles génétiques en nous penchant sur les deux phases de dispersion de l'insecte.

Au travers de la modélisation des abondances, nous avons montré que lors de leur sortie d'hivernation, les méligèthes peuvent parcourir en moyenne 1,2 km. Avant ce travail de thèse, la dispersion des méligèthes était un point assez vague de par le peu d'étude l'estimant. Une étude de capture-marquage-recapture en utilisant des éléments radioactifs avait montré que, à la sortie de l'hiver, les méligèthes pouvaient parcourir 200 m en 2 heures et jusqu'à 12 000 m en 2 jours (Taimr et al., 1967). Les buffers où les variables paysagères étaient significatifs étaient souvent de l'ordre des 2 000 m (Rusch et al., 2011 et Zaller et al., 2008). A l'aide de notre modèle nous avons montré que la distance moyenne de dispersion des méligèthes lors de cette phase était de 1 200 m. Ces données sont en agrément car avec une distance moyenne de dispersion de 1 200 m avec un kernel de dispersion Gaussien ou exponentiel, quelques individus peuvent aller jusqu'à plus de 10 000 m. Ces données sont aussi confirmées par notre assignation de parentés. Avec les modèles d'apparentement, nous avons montré que des méligèthes pouvaient parcourir 1,6 km entre les bois et le colza, ce qui est proche de la valeur trouvée avec les modèles statistiques.

Nous n'avons pas pu mesurer la distance parcourue lors de la phase de dispersion estivale avec nos échantillonnages d'individus et nos méthodes statistiques. Cependant, nous avons trouvé une paire de pleins frères entre un individu échantillonné à l'émergence dans une parcelle de colza et un second individu dans les parcelles de colza de l'année suivante. Ces deux individus se trouvaient à un peu plus de 2 km l'un de l'autre, ce qui voudrait dire que le cumul des deux dispersions est de 2 km pour cet individu. Or, une seconde étude de capture-marquage-recapture a révélé que les méligèthes de la nouvelle génération, donc du colza vers les sites d'hivernation, pouvaient parcourir 1 à 2 km en moyenne (Stechmann & Schütte, 1976). Cela confirmerait les distances énoncées précédemment, obtenues par nos analyses d'apparentement.

Ainsi à chacune de leur phase de dispersion, un méligèthe parcourt en moyenne 1 à 2 km et un petit nombre d'individus pourrait parcourir des distances de plusieurs dizaines de km.

### **3. Limites de l'étude**

#### ***3.1. Analyses génétiques de l'apparentement entre individus***

Les estimations génétiques de la taille efficace des populations et de la dispersion dépendent en partie de la fiabilité de la détection des individus apparentés. Le nombre d'apparentés détectés chez les populations de méligèthes est faible, et dans une moindre mesure il l'est aussi dans les populations de parasitoïdes. Une des raisons pourrait être que, au vu des grandes tailles de populations, la probabilité de trouver des individus frères est très faible. De plus, l'algorithme

de COLONY utilisé « full likelihood » cherche à minimiser le nombre de groupe d'individus apparentés dans la population analysée ; il agrège des individus dans un groupe dès lors que leurs génotypes multilocus sont compatibles pour être apparentés à ce groupe quand bien même la probabilité d'y être apparenté est négligeable. Ce phénomène peut amener à détecter de faux positifs, qui seraient juste des individus proches génétiquement mais pas forcément apparentés. Une manière de réduire le biais est d'augmenter le nombre de marqueurs et leurs niveaux de polymorphisme. Ici, nous avons sélectionné 12 microsatellites pour les méligèthes. Ce nombre de locus est suffisant pour des études de génétique des populations (Pritchard et al., 2000) mais est un peu faible pour des études d'assignement de parentés (Wang, 2012). Cependant, leur polymorphisme compense leur faible nombre. Pour les parasitoïdes, nous avons développé 16 marqueurs mais qui sont beaucoup moins polymorphes, contrairement aux méligèthes, c'est leur nombre qui compense leur faible polymorphisme. Les erreurs d'assignations peuvent aussi être réduites en augmentant la taille de l'échantillonnage, en particulier dans le cas de populations de très grande taille comme pour les méligèthes. Nous avons montré que la probabilité que deux méligèthes aient le même génome au hasard était de  $9 \times 10^{-5}$  ce qui est une probabilité assez faible et montre que nos assignations de parentés semblent fiables. Pour *T. heterocerus* la probabilité que deux individus partagent le même génome est de  $1,6 \times 10^{-3}$  ce qui montre une fiabilité moins forte pour les estimations de parentés mais qui reste raisonnable.

### ***3.2. Le focus sur une région et un ravageur***

Toute notre étude est basée sur une zone d'étude : l'Eure. Cette zone avait été choisie pour sa diversité de paysages, avec des parties plus complexes que d'autres, avec des proportions de bois et de prairies variées. De plus cette zone était connue pour avoir des abondances de méligèthes élevées avec en moyenne plus de 1 500 adultes de méligèthes par m<sup>2</sup> et un taux de parasitisme allant jusqu'à 60 % en 2009 (Rusch et al., 2011a). Les résultats obtenus 6 ans plus tard dans la même zone d'étude sont sensiblement différents. En effet, nous avons plutôt 140 larves en moyenne par m<sup>2</sup> et un taux de parasitisme moyen de 1.5 %. Ces résultats montrent bien que les abondances de méligèthes sont très variables au cours du temps ce qui correspond au ressenti des agriculteurs rencontrés sur le terrain. Ces abondances très variables pourraient avoir été entraînées par la surabondance de parasitoïdes en 2009 entraînant d'abord une forte diminution de la population de méligèthes elle-même entraînant une chute de la population de parasitoïde elle-même permettant un début de rebond actuel : pour la première fois depuis 2009 des agriculteurs considèrent avoir eu des pertes de rendement liées aux méligèthes en 2017 (communication personnelle de l'ingénieur régional Terres Inovia dans la région). Un suivi

continu de ces deux espèces sur une dizaine d'années serait nécessaire pour éclaircir cette dynamique démographique.

Enfin, nous avons choisi de ne travailler que sur un ravageur et un ennemi naturel. Le colza est soumis à de nombreux autres ravageurs (Alford et al., 2003) et les méligèthes sont eux aussi soumis à un grand nombre d'ennemis naturels (Osborne, 1960). Cependant, une étude exhaustive des ravageurs du colza et de leurs ennemis naturels serait compliquée à mettre en place. Ici, la finalité était de cerner la biologie d'une espèce de ravageur et de son parasitoïde et d'orienter les stratégies de gestion de ce ravageur en fonction des connaissances sur sa biologie.

### **4. Conséquences sur la gestion des méligèthes**

Nos travaux ont apporté des éclaircissements sur la structure génétique des populations de méligèthes, ainsi que sur leur dispersion et la dynamique de population de leur parasitoïde principal en lien avec le paysage. Ces informations ouvrent des perspectives en termes de développement de lutte contre ce ravageur du colza. Ces résultats mettent en avant les caractères spatiaux et temporels de la lutte biologique qui pourrait ou non être appliquée.

La distance moyenne de dispersion des méligèthes a été estimée à environ 1,2 km. En se basant sur ces connaissances, les parcelles de colza pourraient être placées à une distance plus grande des bois pour diminuer les attaques de méligèthes. De plus, comme déjà proposé par d'autres auteurs, des cultures pièges ou des stratégies de type « push pull » pourraient être utilisées. Ainsi, des bandes de navette (*Brassica rapa*) pourraient être placées en tant que plante piège. En effet, cette brassicacée est connue pour être attractive pour les méligèthes, surtout au moment de la floraison (Cook et al., 2004). Cette stratégie permettrait de maintenir les méligèthes hors des parcelles de colza au moment où cette culture est la plus vulnérable, c'est-à-dire en boutons. Cependant, nous avons réalisé des gradients d'abondance de méligèthes dans les parcelles de colza en fonction du temps et de leur distance aux bois, et même si les méligèthes se concentrent dans les parcelles les plus proches des bois, on en trouve quand même dans les parcelles éloignées. Ce point complique l'utilisation de cultures pièges et pourrait être lié au fait qu'une part non négligeable de méligèthes émerge des prairies (Rusch et al., 2011b). Placer des plantes pièges en fonction d'autant d'éléments paysagers (bois, colza, prairies) complique l'utilisation de cette stratégie. Car si le parcellaire de l'agriculteur est éclaté, cette organisation paysagère ne peut se décider que collectivement, a minima entre voisins.



Les méligèthes utilisent aussi les prairies, les bords de bois et de champ ainsi que les jachères comme des ressources entre leur site d'hivernation et leur site de reproduction et réciproquement. Mieux gérer ces espaces pourrait permettre de réduire les sources de nourriture des méligèthes à des moments critiques de leur cycle de vie. C'est-à-dire au moment où ils font des ressources pour l'hiver et au moment où ils sont faibles après l'hiver. Cependant, faucher ces espaces est peut-être à double tranchant car ils sont aussi des sources de nourriture pour leurs ennemis naturels et ceux de d'autres ravageurs ainsi que pour des pollinisateurs sauvages ou domestiques.

Les méligèthes sont attirés par les boutons et les fleurs de colza. Une pratique courante est de mettre quelques graines de colza précoce parmi du colza à floraison normale pour que les méligèthes se concentrent dessus. D'autres agriculteurs sèment des parcelles de colza précoce entières. Ainsi, la période de vulnérabilité du colza est évitée et les méligèthes se reproduisent quand même mais sans faire beaucoup de dégâts. Cette solution pose des problèmes quant au cycle des parasitoïdes. En effet, les parasitoïdes émergent déjà tard dans le cycle du colza, en mai alors que le colza commence à faner et les larves de méligèthes à s'enfouir dans le sol pour entrer en nymphose. Si on sème plus de variétés précoces, on va encore accroître cet écart, au risque que les parasitoïdes et entre autres *T. heterocerus*, émergent après la fin de la floraison du colza et n'aient plus d'impact sur les méligèthes.

Comme décrit précédemment, les méligèthes dépendent de nombreux habitats, dont certains rarement cités. Ainsi les méligèthes sont connus en tant que ravageurs du colza et hivernant dans les bois ou dans les prairies, puis attaquent le colza. Nous avons aussi montré qu'entre leur étape d'hivernation et la reproduction sur les colzas ils pouvaient utiliser les habitats semi-naturels. Enfin, en été, après avoir émergé du colza, les méligèthes vont se nourrir du pollen des adventices présentes dans les parcelles de colza mais aussi dans les parcelles cultivées avec d'autres cultures. De plus, les parasitoïdes émergent au printemps de l'année suivant le colza, et peuvent être impactés par les pesticides et le travail du sol qui peuvent être utilisés dans les parcelles de colza mais aussi dans la parcelle dans laquelle ils vont émerger, qui sont souvent des céréales (Ulber et al., 2010). La survie des parasitoïdes et donc le contrôle biologique des méligèthes, dépend aussi du travail du sol réalisé après le colza et donc pour la mise en place de la culture suivante (Williams, 2006). Il faut garder en tête que l'effet des parasitoïdes se fait ressentir sur les dégâts causés par les méligèthes seulement l'année suivante et sur d'autres parcelles que la parcelle de colza labourée. Tous ces éléments montrent que la gestion des méligèthes doit être pensée sur l'ensemble du paysage et à une échelle temporelle large.

## 5. Perspectives

### 5.1. Améliorer les connaissances sur les effets du paysage et modélisation

Il serait intéressant de poursuivre le travail et notamment sur les processus de dispersion des méligèthes dans différents paysages. Les marqueurs microsatellites développés sur les méligèthes pourraient être utilisés pour une étude de génétique du paysage sur les méligèthes capturés en 2016 dans les 96 parcelles de l'Eure. Ce type d'analyse est souvent utilisé pour mesurer les déplacements d'espèces à conserver (Dileo et al., 2013) et sont aussi appliquées ces dernières années sur des études concernant des insectes ravageurs (Vialatte et al., 2005).

Préciser la dispersion des parasitoïdes pourrait aussi aider à mieux comprendre l'espèce et à aider à sa régulation. Le nombre de parasitoïdes capturés dans l'Eure en 2016 est trop faible pour mener ce type d'étude. Placer des tentes à émergence dans des parcelles de blé en mai, avant leur émergence permettrait de capturer les individus au moment de leur sortie de diapause. Cependant, il faudrait un grand nombre de parcelles de blé, précédées de colza, si possible non labourées (Hanson et al., 2015) pour que ce soit possible, or peu de parcelles le sont. Une autre solution serait d'utiliser du marquage pour suivre les mouvements de prospection des parasitoïdes après leur émergence. Différents types de marquages peuvent être utilisés sur des parasitoïdes comme des colorants, du pollen, des éléments radioactifs ou des protéines (Lavandero et al., 2004). Des tests de marquage en utilisant des bleuets marqués avec un isotope stable ont été réalisés sur des parasitoïdes du puceron, *Diaeretiella rapae* (Pollier et al., 2016). Ces plants ont été placés dans des bandes fleuries, ainsi lorsque les parasitoïdes se nourrissent du nectar de ces colzas ils sont marqués avec cet isotope. Une fraction de 1 % des parasitoïdes trouvés proches de ces bandes fleuries étaient marqués. Des tests similaires sur *Tersilochus heteroceris* pourraient être réalisés.

Nous avons montré que les méligèthes et les parasitoïdes utilisent de nombreux éléments du paysage. Comme il a été évoqué dans l'introduction de ce travail, un modèle, Mosaic-Pest a été construit sur l'interaction méligèthes, parasitoïdes, paysages (Vinatier et al., 2012a). Ce modèle décrit l'évolution des dommages causés par les méligèthes en fonction de leur abondance, de celle des parasitoïdes et de la composition du paysage. Une analyse de sensibilité de ce modèle avait montré que les dommages réalisés par les méligèthes étaient dépendants de nombreux paramètres tels que la dispersion de cette espèce (Vinatier et al., 2012b). Avec notre nouvelle estimation de la distance de dispersion des méligèthes en sortie d'hivernation, le modèle pourrait être re-paramétré pour estimer l'effet du paysage sur les abondances de méligèthes et leurs dommages. De plus la modélisation employée ne considérait pas que les méligèthes

puissent être attractifs pour les parasitoïdes, un point qui pourrait être intégré aux analyses de sensibilité sur la dispersion des parasitoïdes. Ce type de modèle a entre autre pour objectif d'aider à visualiser les conséquences des changements de pratiques qui seraient conduits sur l'ensemble du paysage pour faciliter la coordination des différents acteurs du paysage. Il est donc important que de tels outils soient correctement calibrés, en ce sens une caractérisation plus précise des distances pouvant être parcourues par les parasitoïdes serait nécessaire.

### **5.2. Autres ravageurs du colza et application des méthodes utilisées contre les méligèthes**

Ces dernières années les parcelles de colza ont été soumises à des attaques de d'autres ravageurs qui ont fait beaucoup de dégâts. C'est notamment le cas des altises du colza, *Psylliodes chrysocephala*. Cet insecte attaque les plantules en automne à la levée, ce qui peut totalement détruire la culture. Les larves attaquent les pieds, ce qui entraîne un retard de la montaison comparé aux pieds sains et une baisse de rendement. Cette espèce fait des dégâts depuis de nombreuses années et son comportement a commencé à être étudié dans les années 80 (Blight et al., 1989). L'augmentation de ces attaques devient problématique pour les rendements du colza. Cependant, seule l'utilisation de pesticides face à cet insecte est pour l'instant une voie possible, bien qu'ils deviennent moins efficaces avec l'apparition de résistances ces dernières années (Zimmer et al., 2014). Certaines des méthodes de lutte utilisées contre les méligèthes peuvent être appliquées à cette espèce. Ainsi, l'utilisation de navette comme plante piège semble efficace contre cette espèce au printemps (Barari et al., 2005). Cependant ce système ne peut fonctionner en automne, ces plantes ne fleurissant pas à cette période. Il serait intéressant de trouver une autre brassicacée se semant en automne et détournant les altises des colzas. La régulation biologique de cette espèce pourrait aussi être améliorée. Une espèce de parasitoïdes, *Tersilochus microgaster*, est connue pour parasiter les larves d'altise (Barari et al., 2005).

Les problématiques liées aux altises au printemps sont les mêmes pour le charançon de la tige (*Ceutorhynchus pallidactylus*) excepté que les plantes pièges n'ont pas d'effet sur lui (Barari et al., 2005). Cette espèce est aussi régulée par des espèces de parasitoïdes du genre *Tersilochus* (Jourdeuil, 1960).

Ces deux ravageurs du colza étant, tout comme les méligèthes, régulés par des parasitoïdes du genre *Tersilochus*, les connaissances liées à l'alimentation de ces ennemis naturels et de leurs déplacements dans le paysage restent des points importants à approfondir pour améliorer la régulation biologique du parasitoïde et réduire les dégâts des altises sur le colza.

***5.3. La complexité de la lutte biologique par conservation : de nombreux acteurs impliqués***

Le colza est une culture dont les rendements sont diminués par de nombreux ravageurs comme nous l'avons montré. La lutte biologique par conservation est complexe et implique de s'intéresser aux différents éléments paysagers qui sont utilisés par les ennemis naturels mais aussi par les ravageurs. Le rôle des espaces semi-naturels évolue sans cesse dans les mentalités. En effet, dans les années 50, ces habitats étaient considérés comme étant des sources de ravageurs (Van Emden, 1964) et la réduction de ces espaces a explosé avec l'accroissement des paysages simplifiés. Dans les années 60, un nouveau mouvement est apparu en comprenant que les habitats semi-naturels pouvaient abriter des ennemis naturels des ravageurs (Van Emden, 1964). Dans notre étude, nous reprenons le fait que les ravageurs et les parasitoïdes dépendent des habitats semi-naturels mais qu'ils dépendent aussi des autres cultures et des zones urbanisées avec les fleurs ornementales. Tous ces points soulignent que la gestion des méligèthes, mais aussi des ravageurs du colza, de leurs ennemis naturels ainsi que celle des ravageurs des autres cultures doit être pensée à l'échelle du paysage et certainement du territoire. De plus, puisque certains ravageurs ont plusieurs périodes d'attaque ou des périodes d'attaques longues et que les parasitoïdes, ainsi que d'autres ennemis naturels ont un effet sur les populations de ravageur de l'année suivante, le paramètre temporel est à prendre en compte. Ces aspects spatio-temporels font bien ressortir que le contrôle biologique des ravageurs devrait être pensé à l'échelle du paysage et sur plusieurs années mais surtout de manière concertée entre différents acteurs du paysage ou du territoire (agriculteurs, éleveurs, jardiniers, communes).

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## Liste des publications et communications

### Publications à comité de lecture réalisées lors de la thèse

Juhel, A. S., Barbu, C. M., Franck, P., Roger-Estrade, J., Butier, A., Bazot, M., & Valantin-Morison, M. (2017). Characterization of the pollen beetle, *Brassicogethes aeneus*, dispersal from woodlands to winter oilseed rape fields. *PloS one*, 12(8)

Juhel, A.S., Barbu, C.M., Valantin-Morison M., Olivares J. & Franck P. (Submitted). Limited genetic structure of *Brassicogethes aeneus* populations in France and in Europe.

Juhel, A.S., Barbu C.M., Valantin-Morison, M., Oliveres, J., and Franck P. (In prep). Using microsatellite markers to improve knowledge on the dispersal of a crop pest parasitoid, *Tersilochus heterocerus*.

Juhel, A.S., Vivet, V., Valantin-Morison, M., Franck P and Barbu C.M., (In prep). The impact of landscape elements on the abundance of a parasitoid is partly related to the abundance of its host.

Juhel, A.S., Barbu C.M, Valantin-Morison, M., Olivares, J. and Franck P (In prep). Sibship assignments and effective population size measure of an oilseed rape pest.

Juhel, A.S., Valantin-Morison M., Franck P., Butier A., Bazot M. and Barbu C.M. (In prep). Wild flowers, important pollen source for an oilseed rape pest. on the dispersal of a crop pest parasitoid, *Tersilochus heterocerus*.

### Présentations lors de conférences

Juhel A., Barbu C.M., Franck P., Butier A., Roger-Estrade J., Valantin-Morison M. (2016). Variation in abundance of pollen beetle, *Meligethes aeneus* and its parasitoid, *Tersilochus heterocerus* in oilseed rape in relation to proximity to woodlands and grasslands. Prospects and progress for sustainable oilseed crop protection 7 –9 September 2016, Tartu (Estonia)

## Liste des publications

### **Posters scientifiques**

Juhel A, Barbu, C., Valantin-Morison, M., Franck, P., Butier, A., Roger-Estrade, J. (2016).

Landscape effect on abundances of pollen beetles and their main parasitoids. ABIES Days. 14-15 Avril, 2016, Paris.

Juhel A, Barbu, C., Franck, P., Vivet, V., Butier, A., Roger-Estrade, J., Valantin-Morison, M..

(2016). Flowers: when, where and which are important for pollen beetles? International Conference on Ecological Science, 24 octobre 2016



**Titre :** Dynamique des populations de méligèthes, *Brassicogethes aeneus* Fabr. (Coleoptera, Nitidulidae) et de son principal parasitoïde, *Tersilochus heterocerus* Thomson (Hymenoptera, Ichneumonidae) en fonction de l'hétérogénéité des paysages agricoles.

**Mots clés :** Dispersion, lutte biologique, méligèthes, parasitoïdes, paysage

**Résumé :** Une régulation biologique plus efficace des ravageurs des grandes cultures par leurs ennemis naturels nécessite une meilleure compréhension de la biologie de ces espèces et de leurs patrons de dispersion dans les paysages agricoles. L'objectif de ce travail est d'améliorer les connaissances sur la dynamique des populations de méligèthes et de leur parasitoïde principal. À l'aide de microsatellites, nous avons montré que la structuration génétique des populations de méligèthes était faible en Europe, celle de *T. heterocerus* est sensiblement plus forte. Avec des modèles statistiques appliqués aux abondances de méligèthes, nous avons montré qu'ils parcourent en moyenne 1,2 km après l'hivernation. Cette distance moyenne de dispersion est comparable à celle estimée à partir de résultats d'assignation de parentés génétique

entre paires d'individus. Avec des relevés de terrain, nous avons quantifié et identifié les déterminants de la présence de méligèthes dans d'autres habitats que le colza. Au printemps, ils peuvent être observés dans des prairies, des friches et des bords de champs, où se trouvent des fleurs jaunes. En été, les méligèthes sont présents dans ces habitats, partout où il y a des fleurs, sans distinction de couleurs, surtout sur les adventices des cultures. Enfin, la présence de parasitoïdes semble plus fortement déterminée par la présence de méligèthes que par des éléments paysagers. Le paysage joue un rôle déterminant sur ce couple d'espèces. De plus, les estimations des paramètres démographiques réalisées pourront aider par la modélisation à dimensionner les actions à mener pour limiter les dégâts causés par les méligèthes.

**Title:** Dynamic of populations of *Brassicogethes aeneus* Fabr. (Coleoptera, Nitidulidae) and of its main parasitoid, *Tersilochus heterocerus* Thomson (Hymenoptera) depending on the heterogeneity of the landscape

**Keywords:** Biological control, dispersal, landscape, parasitoids, pollen beetles

**Abstract:** More effective biological regulation of field crop pests by their natural enemies requires a better understanding of the biology of these species and their patterns of dispersal in agricultural landscapes. The objective of this work is to increase knowledge on the dynamics of pollen beetles populations and their main parasitoid. Using an approach based on the analysis of microsatellites, we have shown that the genetic structuring of pollen beetle populations in Europe is weak. Populations of *T. heterocerus* are more structured. With statistical models applied to the abundance of pollen beetles, we have shown that they travel an average of 1.2 km, after overwintering. This average distance is comparable to that estimated from results of sibship analysis between pairs of individuals

With fieldwork, we quantified and identified the determinants of pollen beetles presence in habitats other than rapeseed. In spring, pollen beetles can be seen in grasslands, fallows and field edges with yellow flowers. In summer, pollen beetles are present in these habitats, wherever there are flowers, without distinction of colour, especially on the weeds of crops. Finally, the presence of parasitoids seems to be more strongly determined by the presence of pollen beetles than by landscape elements. The landscape plays a decisive role on this pair of species. Moreover, through modelling, estimates of the demographic parameters carried out would help to shape the actions to be taken to limit the damage caused by pollen beetles.