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**Des odeurs pour protéger les cultures : utilisation de  
composés volatils pour modifier le comportement de la  
mouche du chou, *Delia radicum* et de ses ennemis  
naturels**

Alan Kergunteuil

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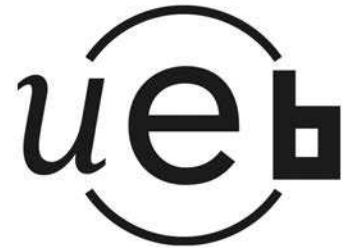
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ANNEÉ 2013



**THÈSE / UNIVERSITÉ DE RENNES 1**  
*sous le sceau de l'Université Européenne de Bretagne*

pour le grade de  
**DOCTEUR DE L'UNIVERSITÉ DE RENNES 1**  
*Mention : Biologie*

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présentée par

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Préparée à l'UMR 1349 « IGEPP »  
Institut de Génétique, Environnement et Protection des Plantes  
UFR Sciences de la Vie et de l'Environnement

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**Des odeurs pour  
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pour modifier le  
comportement de la  
mouche du chou,  
*Delia radicum* et de  
ses ennemis naturels**

**Thèse soutenue à Rennes  
le 28 Novembre 2013**

devant le jury composé de :

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En argot  
les hommes appellent les oreilles  
  des feuilles  
c'est dire comme ils sentent  
que les arbres connaissent la musique  
Mais la langue verte des arbres  
est un argot bien plus ancien  
  qui peut savoir ce qu'ils disent  
  lorsqu'ils parlent des humains

Les arbres parlent arbre  
comme les enfants parlent enfant

Quand un enfant  
  de femme et d'homme  
adresse la parole à un arbre  
  l'arbre répond  
  l'enfant entend

Plus tard  
  l'enfant parle arboriculture  
  avec ses maîtres et ses parents  
Il n'entend plus la voix des arbres  
  il n'entend plus  
  leur chanson dans le vent ...

Jacques Prévert (Arbres)



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A mes parents et à mes frères,

A Flora.

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# **INTRODUCTION GENERALE**



# 1. Les réseaux trophiques dans les agrosystèmes

Au sein d'une communauté écologique, un réseau trophique définit comment les espèces sont reliées entre elles par des interactions de type proies-prédateurs. L'organisation des communautés en réseaux trophiques permet alors de comprendre le fonctionnement d'un écosystème à travers plusieurs notions liées à l'équilibre d'un milieu biologique (*e.g.* résistance, résilience) (Pascual & Dunne, 2006). Bien qu'un système trophique puisse comprendre un grand nombre d'espèces, il est possible de les regrouper en fonction des proies et des prédateurs partagés par chaque espèce (Sugirha *et al.*, 1989 ; Pimm *et al.*, 1991). Dans le cadre des relations plantes – insectes, les réseaux tri-trophiques se composent ainsi de trois niveaux distincts : les producteurs primaires (les plantes), les consommateurs primaires (les herbivores) et les consommateurs secondaires (les ennemis naturels des herbivores) (Price *et al.*, 1980). La structure d'un réseau trophique et la stabilité qui en résulte reposent en grande partie sur des équilibres dynamiques (initialement formulés à travers l'équation de Lotka-Volterra) contrôlant les populations de chaque organisme impliqué (Cohen *et al.*, 1990). Plusieurs facteurs environnementaux influencent ces équilibres (indépendamment des relations de prédation, de compétition ou de mutualisme) et contribuent à l'architecture du système trophique (Jenkins *et al.*, 1992 ; Ripa *et al.*, 1998). Les agrosystèmes sont des milieux particuliers subissant des perturbations de manière fréquente et régulière. La simplification de l'habitat et l'implantation de monocultures favorisent la colonisation des parcelles par les insectes ravageurs (Meehan *et al.*, 2011). Parallèlement, une rapide succession d'habitats provoque un phénomène d'asynchronie où la colonisation de la parcelle par les herbivores en début de saison a lieu en l'absence de leurs ennemis naturels (Southwood, 1977). Dans les deux cas, les dégâts occasionnés par les herbivores proviennent de déséquilibres au sein de la structure des systèmes trophiques. Dans les agrosystèmes, les mécanismes intervenant dans les équilibres des réseaux tri-trophiques et la régulation des populations d'herbivores ont donc suscité beaucoup d'intérêt.

Tout d'abord, le développement des phytophages dépend des ressources auxquelles ils ont accès et les populations sont donc régulées par la qualité et la disponibilité des plantes présentes dans l'environnement. Le contrôle des herbivores s'effectue ainsi d'un niveau trophique inférieur vers un niveau supérieur (contrôle « bottom-up ») (Newton *et al.*, 2009;

Blake *et al.*, 2010). Les populations de phytophages sont également régulées par leurs ennemis naturels. Le contrôle des herbivores s'effectue cette fois-ci d'un niveau trophique supérieur vers un niveau inférieur (contrôle « top-down ») (Gomez & Zamora, 1994 ; Halaj & Wise, 2001). Ces deux mécanismes de contrôle des populations d'herbivores ne sont pas nécessairement exclusifs et peuvent tous les deux être intégrés dans les systèmes de défenses mis en place par les plantes contre leurs agresseurs, qu'ils soient directs (« bottom-up ») ou indirects (« top-down ») (Ode, 2006). Ces deux types de contrôle sont à l'origine des deux hypothèses écologiques proposées par Root (1973) pour expliquer pourquoi les populations d'herbivores sont plus importantes dans les monocultures que dans des habitats naturels plus diversifiés. Dans un habitat diversifié où les ressources sont moins concentrées, les insectes phytophages ont plus de difficultés à trouver une plante hôte, ce qui réduit la densité de leurs populations via un effet de type « bottom-up » (hypothèse de la concentration des ressources). Par ailleurs, l'effet « top-down » exercé par les ennemis naturels sera plus important dans un habitat complexe capable de les accueillir et de favoriser leur action (hypothèse des ennemis naturels).

De manière générale, ces mécanismes de régulation des populations d'herbivores reposent sur des connections entre deux ou plusieurs niveaux trophiques. L'écologie des communautés est longtemps resté le seul cadre scientifique à l'intérieur duquel les chercheurs ont essayé de comprendre ces connections. Plus récemment, certains auteurs ont souligné l'intérêt d'une approche centrée sur les comportements individuels de recherche de nourriture (Krivan & Schmitz, 2003, Mc Cann *et al.*, 2005). En effet, la capacité des organismes impliqués dans un réseau trophique à localiser des ressources définit en partie leurs régimes alimentaires. Ces traits comportementaux sont donc à la base des connections entre niveaux trophiques et contribuent ainsi à caractériser la structure d'un réseau (Beckerman *et al.*, 2006). L'écologie chimique est une discipline qui étudie le rôle des signaux chimiques dans les communications entre des individus émetteurs et récepteurs d'informations. Cette discipline représente ainsi un moyen d'appréhender l'organisation des réseaux tri-trophiques (et les équilibres sous-jacents) où les espèces de chaque niveau peuvent à tour de rôle émettre un message ou le recevoir avant d'adapter leur réponse. L'utilisation de ces connaissances en agriculture pourrait permettre de développer de nouveaux moyens de protection des cultures.

## 2. L'organisation des réseaux trophiques repose sur l'échange d'information

Un réseau trophique est un réseau où circulent des informations utilisées par les organismes pour communiquer entre eux. Une communication biologique implique un organisme émettant une information et un organisme récepteur détectant cette information au niveau du système nerveux périphérique avant de la transformer en signal au niveau du système nerveux central (processus de traduction). L'organisme récepteur déclenche alors une réponse lui permettant d'adapter son comportement. La définition d'une communication biologique peut ainsi se référer à une approche adaptative ou à une approche centrée sur l'échange d'information (Scott-Phillips, 2008). Bien qu'une information puisse être véhiculée sous différentes formes (*e.g.* visuel, sonore), celles de nature chimique sont certainement les plus répandues (Steiger *et al.*, 2011). Dans le cadre des communications inter-spécifiques, les substances sémi-chimiques transportant l'information peuvent être catégorisées en fonction de l'effet dû à la réponse de l'organisme récepteur sur les deux organismes impliqués dans la communication : (i) les allomones sont favorables uniquement à l'organisme émetteur (ii) les kairomones sont favorables uniquement à l'organisme récepteur (iii) les synomones sont favorables à l'organisme émetteur et à l'organisme récepteur (Nordlund & Lewis, 1976).

### 2.1. Les herbivores utilisent un ensemble de signaux pour sélectionner une plante hôte

Loin d'être une exception, la spécialisation des insectes herbivores semble être une généralité : moins de 10% d'entre eux se nourrissent sur plus de trois familles de plantes (Benays & Graham, 1988). Cette spécialisation nécessite une sélection efficace de la plante hôte. La séquence comportementale impliquée dans cette sélection se divise en trois grandes phases : la localisation, la reconnaissance et l'acceptation de la plante hôte. Afin d'optimiser une ou plusieurs de ces étapes, l'insecte utilise principalement trois types de stimuli d'origine végétale : olfactifs, visuels et gustatifs. La plupart des séquences comportementales font appel à ces trois stimuli bien que plusieurs caractéristiques spécifiques de l'herbivore (*e.g.* rythme circadien, mode de locomotion et degré de spécialisation) puissent modifier leurs importances relatives (Schoonhoven *et al.*, 2005).

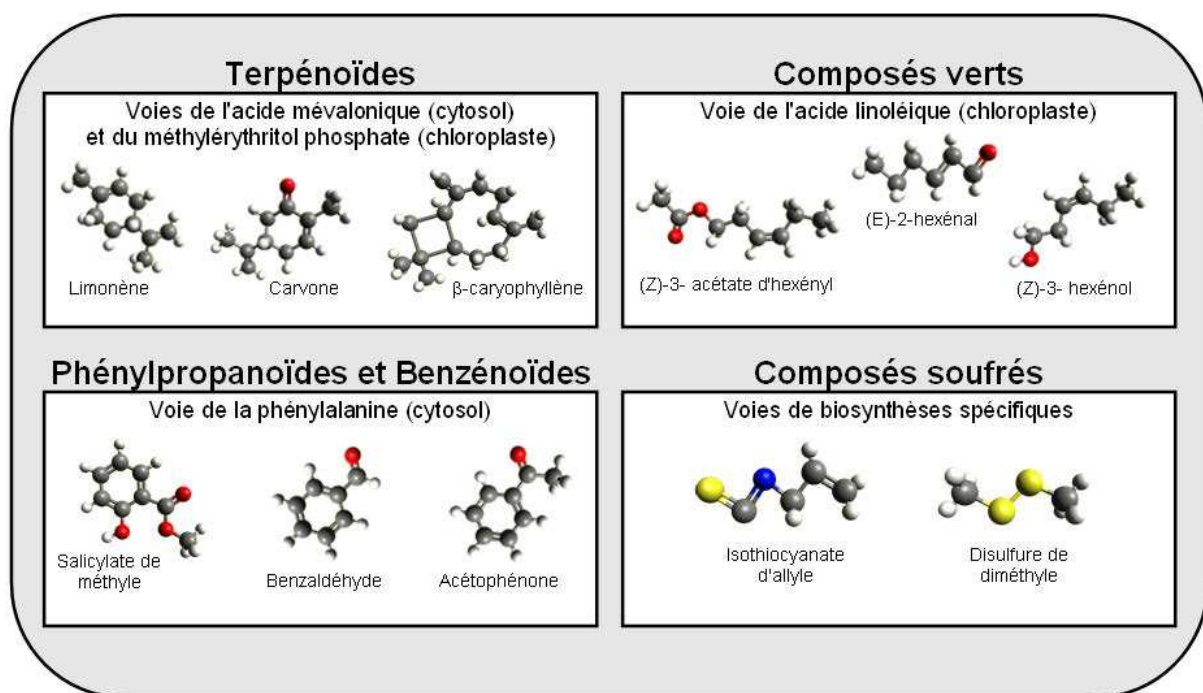
À distance, l'herbivore utilise des stimuli visuels et olfactifs pour localiser une plante hôte. Les stimuli visuels permettent la caractérisation d'une plante à plusieurs niveaux. Ainsi la couleur, la forme et la surface foliaire ou encore la hauteur de la plante et son isolement par rapport aux plantes voisines peuvent être utilisés par l'insecte comme source d'information (Prokopy & Owens, 1983). Les stimuli visuels sont fortement directionnels (*i.e.* ils permettent de s'orienter très précisément) mais relativement peu informatifs quant à l'espèce végétale. À l'inverse, les signaux olfactifs peuvent être peu directionnels (changement de direction du vent, de sa vitesse) mais beaucoup plus spécifiques (cf. I.2.2.2.). Bien qu'une chimiotaxie soit difficile à maintenir dans une atmosphère turbulente, une anémotaxie positive conditionnée par des odeurs de plantes hôtes permet à l'insecte de s'affranchir partiellement des contraintes dues à ce milieu de diffusion (Visser, 1986; Schoonhoven *et al.*, 2005). Pour déchiffrer les signaux chimiques qui abondent dans leur environnement, les insectes sont munis de récepteurs olfactifs qui leur permettent de percevoir les odeurs émises par leur plante hôte (cf. I.2.2.2.). Des composés de plantes non hôtes peuvent également être détectés et permettre à l'herbivore d'éviter des plantes inappropriées (Nottingham *et al.*, 1991 ; Pickett *et al.*, 2012). Ainsi chez certains herbivores comme les scolytes, une utilisation séquentielle d'un ratio de stimuli olfactifs issus de plantes hôtes et de plantes non-hôtes permet de localiser un habitat convenable, une espèce hôte et enfin une plante hôte de bonne qualité (Zhang & Schlyter, 2004).

La phase de localisation prend fin dès l'atterrissage de l'insecte sur la plante. Au contact de la plante, l'herbivore a alors accès à d'autres stimuli pour reconnaître et accepter une plante hôte. Plusieurs facteurs physiques comme la texture (Roessingh & Städler, 1990) et la présence de trichomes (Calatayud *et al.*, 2008; Firdaus *et al.*, 2012) peuvent influencer la décision de rester sur la plante et moduler l'oviposition. Cependant, après atterrissage la majorité de l'information spécifique se trouve, là encore, sous forme de signaux chimiques bien qu'elle soit cette fois-ci constituée de stimuli gustatifs perçus par des récepteurs de contact. La grande diversité des métabolites secondaires présents à la surface des feuilles permet en effet de reconnaître efficacement une plante hôte. Les profils biochimiques détectés par l'insecte forment une « empreinte » composée de stimuli gustatifs positifs (stimulants) et négatifs (inhibiteurs) au sein de laquelle de légères différences peuvent modifier la nutrition et l'oviposition (Jermy, 1984, Städler *et al.*, 2002). Plusieurs métabolites tels que les glucosinolates, les flavonoïdes et les alcaloïdes peuvent par exemple stimuler ou inhiber ces comportements en fonction du degré de spécialisation de l'herbivore (Schoonhoven *et al.*,

2005). La reconnaissance de la plante hôte repose donc principalement sur des stimuli gustatifs même si plusieurs exemples montrent que l'oviposition peut être modulée par des effets synergiques entre des stimuli gustatifs, olfactifs et quelque fois visuels (Roessingh & Städler, 1990 ; Justus & Mitchell, 1996 ; de Jong & Städler, 1999 ; Tasin *et al.*, 2011).

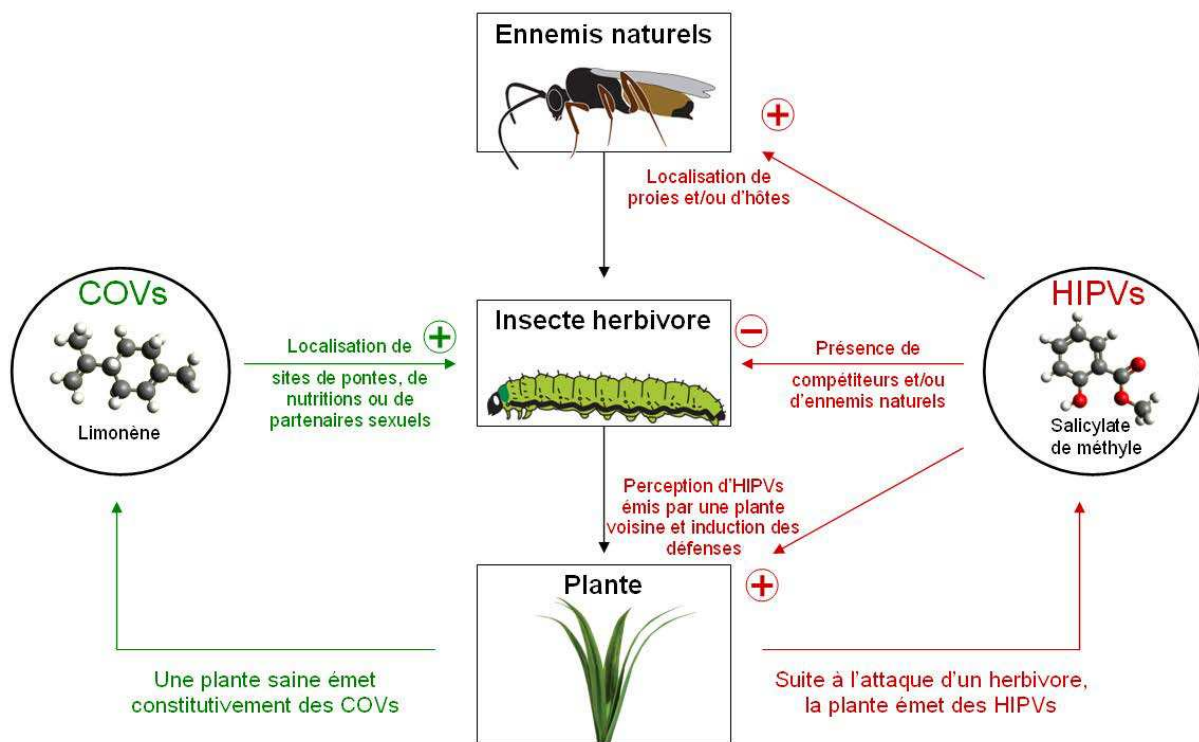
## 2.2. Fonctions écologiques des odeurs de plantes au sein des réseaux tri-trophiques

Toutes les plantes libèrent dans leur environnement un mélange de composés organiques volatils (COVs) à travers plusieurs organes comme les fleurs, les feuilles, les racines et certains tissus spécialisés (*e.g.* trichomes) (Holopainen & Blande, 2012). A ce jour plus de 1700 COVs ont été identifiés et ce chiffre devrait augmenter avec les progrès réalisés dans les méthodes analytiques (Dudareva *et al.*, 2006). Il est possible de regrouper l'ensemble de ces COVs en quatre grandes familles en fonction de leurs natures chimiques et de leurs voies de biosynthèses (*cf.* figure 1). La complexité d'un bouquet d'odeurs constitué de plusieurs centaines de COVs émis simultanément par une plante (Fraser *et al.*, 2003 ; Gaquerel *et al.*, 2009) est à l'origine d'une grande diversité d'informations pouvant être perçues par les insectes (Bruce *et al.*, 2005 ; Bruce & Pickett, 2011). Les différentes fonctions écologiques assurées par les COVs à chacun des niveaux d'un réseau tri-trophique sont présentées dans la partie suivante ; elles sont également résumées dans la figure 2.



**Figure 1 :** Les quatre grandes familles de composés organiques volatils et leurs voies de biosynthèse





**Figure 2 :** Fonctions écologiques des composés organiques volatils (COVs) et des composés volatils induits (HIPVs) au sein d'un réseau tri-trophique

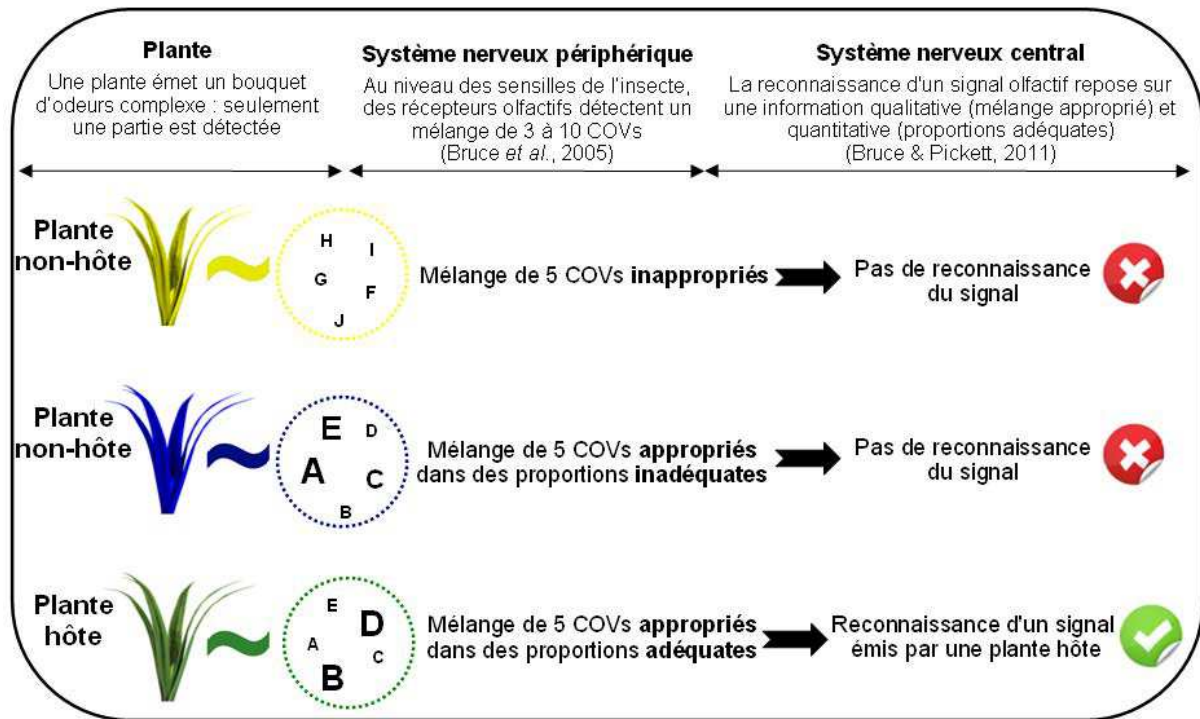
### 2.2.1. Communication plante – plante

En raison de leur enracinement, les plantes sont caractérisées par une vie fixée où les signaux volatils sont cruciaux pour communiquer entre elles. Le rôle des tissus conducteurs dans l'induction systémique des défenses suite à l'attaque d'un herbivore, a été bien décrit (Stramann, 2003). Cependant, ce mécanisme peut être limité par des contraintes liées à l'architecture vasculaire de la plante (*e.g.* phyllotaxie, connections des tissus conducteurs entre plusieurs organes). A l'échelle d'une même plante, les COVs émis par une feuille attaquée permettent de dépasser ces contraintes et d'induire une réponse (*e.g.* sécrétion de nectar extra-floral) chez des feuilles voisines (Orians, 2005 ; Heil & Bueno, 2007). Cette communication privée au sein d'une même plante est sans doute le précurseur des communications entre différentes plantes (Heil & Karban 2010; Steiger *et al.*, 2011). En effet, des plantes saines peuvent percevoir des composés volatils induits par l'attaque d'herbivores (aussi appelés HIPVs pour « herbivore induced plant volatiles ») chez des plantes avoisinantes pour préparer et améliorer leur propre arsenal défensif en prévision d'une possible attaque (Holopainen & Blande, 2012). Cette information peut être « mémorisée »

sous forme de méthylation génétique et accélérer par exemple la synthèse d'inhibiteur de protéases lorsque l'attaque est effective (Ali *et al.*, 2013). De telles communications peuvent avoir lieu entre des plantes appartenant ou non à la même espèce et permettre à la plante induite de limiter les dégâts occasionnés par l'herbivore au moment de l'attaque (Engelberth *et al.*, 2004; Kessler *et al.*, 2006; Ton *et al.*, 2006).

### 2.2.2. Communication plante – herbivore

Plusieurs hypothèses ont été proposées pour expliquer la détection et la reconnaissance d'un signal olfactif par un insecte herbivore. Ces différentes hypothèses ne sont pas nécessairement exclusives et plusieurs scénarios sont observables dans la nature. Deux premières hypothèses ont été formulées à la fin des années 80 : *i/* la reconnaissance d'un signal peut reposer sur la détection de COVs spécifiques d'un taxon ou *ii/* sur celle d'un mélange de COVs ubiquistes émis dans des proportions spécifiques (*cf.* figure 3) (Visser, 1986). Bien que la première hypothèse soit longtemps restée prédominante dans la littérature, de plus en plus d'exemples viennent renforcer la seconde. Des études récentes ont d'ailleurs permis d'affiner l'hypothèse de la reconnaissance d'un signal à partir d'un mélange de composés communs compris entre 3 et 10 COVs (Bruce *et al.*, 2005). La co-localisation de plusieurs récepteurs au sein d'une même sensille permet une résolution spatio-temporelle ; l'insecte est ainsi capable de distinguer un mélange approprié émis par une seule et même plante hôte (et transporté sous forme d'un paquet d'odeurs homogène), d'un mélange approprié résultant d'une combinaison de COVs émis par plusieurs plantes non-hôtes (Bruce *et al.*, 2005). En constatant de légères différences au sein des profils de COVs émis par plusieurs plantes appartenant au même génotype, une étude a suggéré que le système olfactif des insectes herbivores présente une certaine plasticité leur permettant de s'accommoder de telles variations sans pour autant perdre leur capacité à discriminer une plante hôte d'une plante non-hôte (Bruce & Pickett, 2011 ; Riffell, 2012). Au sein d'un bouquet d'odeurs complexe, un signal olfactif est alors constitué d'un ou plusieurs COVs indispensables et de composés volatils pouvant être substitués par d'autres COVs émis par la plante hôte.



**Figure 3 :** Reconnaissance d'un signal olfactif par un insecte herbivore à partir de composés organiques volatils (COVs) communs  
 Les différents COVs détectés et leurs proportions respectives sont schématisés par des lettres et des tailles différentes.

Par ailleurs, les variations des profils de COVs émis par une même plante peuvent également représenter pour l'insecte herbivore une source d'information additionnelle lui permettant d'améliorer ses prises de décisions. Le profil de COVs émis par une plante est en effet susceptible de se modifier au cours de son développement. De telles variations sont utilisées par certains herbivores se nourrissant sur un stade phénologique particulier pour optimiser la localisation d'une plante hôte appropriée (Bengtsson *et al.*, 2001; Szendrei *et al.*, 2009; Magalhães *et al.*, 2012). Les composés volatils produits par une plante sont également sous l'influence de nombreux stress biotiques et abiotiques susceptibles de modifier l'odeur réellement émise (Holopainen & Gershenson, 2010). Ces variations peuvent informer l'herbivore sur la qualité de la plante et lui permettre d'ajuster son comportement (Pinto *et al.*, 2010; Piesik *et al.*, 2013). Les odeurs émises par des plantes attaquées peuvent aussi servir à éviter des plantes accueillant déjà d'autres herbivores, potentiellement compétiteurs, ou des prédateurs (Sabelis *et al.*, 2001 ; Choh & Takabayashi, 2007). Au-delà d'un site de nutrition ou d'oviposition, une plante hôte peut également constituer un site de rencontre. La détection de COVs permet ainsi d'optimiser l'utilisation de signaux phéromonaux chez plusieurs herbivores (Saïd *et al.*, 2005 ; Dickens, 2006; von Arx *et al.*, 2012).

### 2.2.3. Communication plante - ennemis naturels

Les plantes perçoivent très rapidement l'attaque d'un herbivore, que ce soit lorsqu'il se nourrit ou au moment où il pond (Hilker & Meiners, 2010). Dans de nombreux cas les plantes répondent par l'émission d'HIPVs pouvant être synthétisés *de novo* ou être issus de composés membranaires ayant été dégradés suite à l'attaque (Arimura *et al.*, 2005). La découverte du rôle de ces composés volatils dans le recrutement de prédateurs ou de parasitoïdes remonte à une vingtaine d'années (Dicke & Sabelis, 1988 ; Turlings *et al.*, 1990). Ce mécanisme de défense indirecte a fait l'objet de nombreuses études (principalement sur des plantes d'intérêt agronomique) jusqu'à aujourd'hui. Récemment, Mumm & Dicke, 2010 ont recensé près de 50 espèces de plantes capables de recruter des ennemis naturels appartenant à 5 ordres différents : Hymenoptera, Neuroptera, Diptera, Coleoptera et Heteroptera. Dans certains cas, l'émission d'HIPVs peut réduire de 90% le nombre d'herbivores présents sur une plante en agissant à la fois sur le recrutement des ennemis naturels et en réduisant l'oviposition du phytophage (Kessler & Baldwin, 2001). Dans un contexte écologique, une même plante est souvent attaquée simultanément par plusieurs espèces d'herbivores. Lors de l'attaque combinée de plusieurs herbivores, les profils d'HIPVs émis par la plante sont différents de ceux libérés suite à l'agression d'un seul de ces herbivores. Il a été montré que des effets synergiques peuvent avoir lieu : l'attraction d'un prédateur généraliste est par exemple renforcée lorsqu'une plante est attaquée par deux espèces d'herbivores (Moayeri *et al.*, 2007). Cependant, un parasitoïde spécialiste peut quant à lui estimer que son hôte est de moins bonne qualité lorsqu'il est en compétition avec une autre espèce d'herbivore présente sur la même plante. Cette perception d'une compétition possible est liée à l'altération du signal attractif originel en cas de multi-herbivorie (Pierre *et al.*, 2011).

## 3. Utilisation des COVs en protection des cultures

Les multiples fonctions écologiques des COVs peuvent être considérées comme des leviers comportementaux intéressants dans le développement de stratégies de protection des cultures contre les insectes ravageurs. Deux revues récentes illustrent les différentes applications agronomiques utilisant des COVs de synthèse pour modifier le comportement de l'herbivore, de ses ennemis naturels ou des deux (Rodriguez-Saona & Stelinski, 2009 ; Reddy & Guerrero, 2010).

## 3.1. Aperçu général de trois stratégies utilisant des COVs de synthèse

### 3.1.1. Piégeage

Les COVs émis par des plantes hôtes sont utilisés pour piéger des ravageurs de différents types de cultures. Au sein de verger de pommiers, des pièges diffusant un ester permettent par exemple de capturer autant de carpocapses (*Cydia pomonella*) que les pièges à phéromone habituellement utilisés et présentent l'avantage de cibler aussi bien les mâles que les femelles (Light *et al.*, 2001). D'autres pièges contenant cette fois-ci un mélange de trois COVs (linalol, cynamaldéhyde et alcool cynamique) permettent la capture de bruches (*Bruchus rufimanus*) s'attaquant aux cultures de pois et aboutissent à l'élaboration d'outils d'aide à la décision (Bruce *et al.*, 2011). Les pièges attractifs utilisant des kairomones de plantes hôtes peuvent aussi être combinés avec un insecticide dans la mise en place d'« attracticides ». Après la découverte de composés volatils attirant les doryphores (*Leptinotarsa decemlineata*), la combinaison de trois de ces COVs avec un pyréthriinoïde a montré des résultats encourageants pour protéger les cultures de pomme de terres (Dickens, 2000 ; Martel *et al.*, 2007). Une équipe de recherche australienne a récemment commercialisé un « attracticide » pour lutter contre des noctuelles du coton. Leur démarche est détaillée dans une série de trois articles présentant les avantages d'un « super-mélange » de COVs ne cherchant pas à imiter un bouquet d'odeur naturellement émis par une plante hôte mais combinant des odeurs caractéristiques de sites de ponte et de sites de nutrition (del Soccoro *et al.*, 2010 a ; Gregg *et al.*, 2010 ; del Soccoro *et al.*, 2010 b)

### 3.1.2 Synergie avec des phéromones

L'utilisation de phéromones pour protéger les cultures est relativement répandue à travers le monde, que ce soit pour détecter la présence d'herbivores, mettre en place des pièges limitant les populations ou en confusion sexuelle (Witzgall *et al.*, 2010). Plusieurs études font état de résultats prometteurs dans la combinaison de phéromones et de COVs. Tandis que quelques exemples indiquent que la présence de COVs peut inhiber la réponse d'un herbivore à un signal phéromonal (*e.g.* Morewood *et al.*, 2003), des effets additifs ou synergiques entre odeurs de plantes et phéromones sont observés chez de nombreux insectes. Un composé commun à de nombreuses plantes comme le (Z)-3- acétate d'hexényl peut

favoriser la réponse de plusieurs herbivores à leurs phéromones sexuelles respectives comme chez certaines noctuelles (*Helioverpa zea*, *Heliothis virescens*), le carpocapse (*C. pomonella*) et la teigne des crucifères (*Plutella xylostella*) (Reddy & Guerreo, 2004 ; Rodriguez-Saona & Stelinsky, 2009). Les odeurs de plantes permettent également d'améliorer l'effet de phéromones agrégatives. Le (E)-2-hexenol multiplie ainsi par deux le nombre de charançons du coton (*Anthonomus grandis*) capturés comparé à un piège contenant seulement la phéromone agrégative (Aldrich *et al.*, 2003).

### 3.1.3 Contrôle biologique

La grande majorité des études s'étant intéressé au potentiel des COVs dans la protection des cultures s'est concentrée sur l'effet de tels signaux dans le recrutement des ennemis naturels et sur le contrôle biologique des ravageurs. Le phénomène d'asynchronie observé dans la colonisation des parcelles par les herbivores et leurs ennemis naturels permet aux premiers de pulluler en début de saison sans subir d'effets « top-down ». L'utilisation de COVs synthétiques permettant de recruter plus rapidement les ennemis naturels peut limiter ce phénomène et améliorer la protection de la parcelle (Kaplan, 2012). Les premières études ayant démontré l'attraction d'ennemis naturels grâce à la diffusion de COVs synthétiques en plein champ ont été menées aux Etats-Unis par le groupe de David James. La diffusion d'odeurs comme le salicylate de méthyle, le (Z)-3-hexenol et le benzaldehyde ont par exemple permis d'augmenter la densité de coccinelles, de syrphes ou de chrysopes dans des vignobles ou des vergers de houblon (James, 2003 ; James & Price, 2004 ; James, 2005). Le salicylate de méthyle est sans doute le COV ayant été le plus étudié. A partir de l'influence de ce composé sur le comportement d'ennemis naturels, une synthèse a recensé 14 publications menées dans 9 cultures différentes. Sur l'ensemble des 91 observations obtenues, les auteurs ont compté 41 cas d'attraction de prédateurs ou de parasitoïdes (Rodriguez-Saona *et al.*, 2011). Bien que l'utilisation de diffuseurs commerciaux ou expérimentaux soit prédominante, certains auteurs ont préféré pulvériser des COVs sur les feuilles. Appliqués sous cette forme dans des cultures de brocoli, de vigne et de maïs, différents COVs tels l'antranilate de méthyle, le jasmonate de méthyle (MeJA) ou (Z)-3- acétate d'hexényle permettent d'attirer plusieurs familles de parasitoïdes comme les Trichogrammatidae, les Braconidae ou les Scelionidae (Simpson *et al.*, 2011).

## 3.2. La stratégie « Push-Pull » ou le détournement stimulo - dissuasif

### 3.2.1. Principes

Le concept d'une stratégie « Push-Pull » apparaît à la fin des années 80 lorsque des chercheurs imaginent pour la première fois qu'une combinaison de stimuli positifs et négatifs puisse être efficace pour lutter contre les noctuelles s'attaquant aux cultures de coton australien (Pyke *et al.*, 1987 cité par Cook *et al.*, 2007). Quelques années plus tard, Miller & Cowles, 1990 profitent de leurs travaux sur la mouche de l'oignon (*Delia antiqua*) pour reprendre ce concept et définir ce qu'ils appellent alors la stratégie de détournement stimulo-dissuasif. Cette nouvelle méthode de protection des cultures repose sur la modification comportementale du ravageur grâce à une utilisation simultanée de stimuli limitant les dégâts occasionnés sur une culture d'intérêt et concentrant l'herbivore sur une zone restreinte de la parcelle. Dans une revue publiée en 2007, Samantha Cook et ses co-auteurs ont synthétisé l'ensemble des travaux ayant contribué au développement de stratégies « Push-Pull » en répertoriant les différents types de stimuli pouvant être utilisés et en soulignant les principaux avantages de ce type de contrôle (Cook *et al.*, 2007). La modification du comportement du ravageur peut se faire à travers une modification de l'habitat et de la diversité végétale présente sur la parcelle ou par l'utilisation de substances sémiocchimiques. Le tableau 1 présente les sept stratégies de « Push-Pull » mises en place à partir de stimuli d'origine végétale, qu'ils soient naturellement émis ou issus de produits de synthèse.

**Tableau 1:** Stratégies de « Push-Pull » utilisant des stimuli d'origine végétale

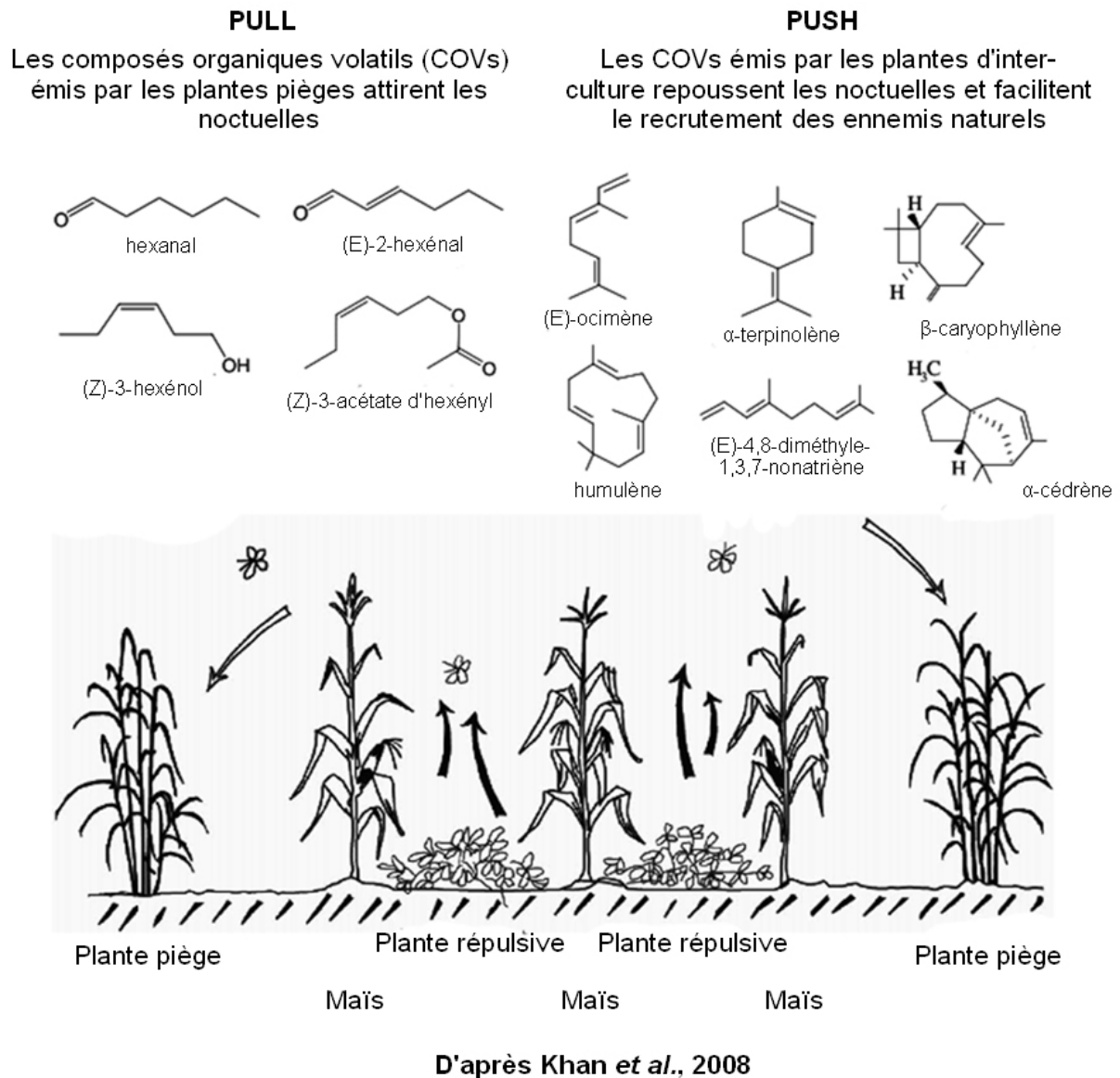
Plante protégée	Ravageur	Stimuli utilisés dans la composante "Push"	Stimuli utilisés dans la composante "Pull"	Références
Coton ( <i>Gossypium hirsutum</i> )	<i>Helicoverpa armigera</i>	extrait de neem ( <i>Azadirachta indica</i> )	plante piège (maïs et pois d'Angole)	Pyke <i>et al.</i> , 1987 cité par Cook <i>et al.</i> , 2007
Oignon ( <i>Allium cepa</i> )	<i>Delia antiqua</i>	cinnamaldéhyde	plante piège (bulbe d'oignons)	Miller & Cowles, 1990
Fève ( <i>Vicia faba</i> )	<i>Sitona lineatus</i>	extrait de neem ( <i>Azadirachta indica</i> )	phéromone agrégative	Smart <i>et al.</i> , 1994 cité par Cook <i>et al.</i> , 2007
Chrysanthème ( <i>Chrysanthemum</i> sp.)	<i>Frankliniella occidentalis</i>	plante répulsive (COVs non identifiés)	plante piège assisté par la diffusion de (E)- $\beta$ -farnésène	Bennison <i>et al.</i> , 2001
Pomme de terre ( <i>Solanum tuberosum</i> )	<i>Leptinotarsa decemlineata</i>	extrait de neem (1) ( <i>Azadirachta indica</i> )	(Z)-3- acétate d'hexényl linalol, salicylate de méthyle (2)	Martel <i>et al.</i> , 2005 a (1) Martel <i>et al.</i> , 2005 b (2)
Pins ( <i>Pinus</i> spp.)	<i>Dendroctonus ponderosae</i>	phéromone anti-agrégative et mélange COVs de plantes non-hôtes	phéromone, myrcène et terpinolène	Giellette & Munson, 2007
Colza ( <i>Brassica napus</i> )	<i>Meligethes aeneus</i>	huile essentielle de lavande (1)	plante piège (2) (navet)	Mauchline <i>et al.</i> , 2013 (1) Cook <i>et al.</i> , 2007 a (2)

La composante « Push » visant à repousser ou à dissuader le ravageur peut par exemple faire appel à des plantes d'interculture perturbant l'orientation de l'herbivore, à des réducteurs d'appétence ou à des phéromones anti-agrégatives (*e.g.* phéromone d'alarme). La composante « Pull » ayant pour objectif de concentrer ou de stimuler l'herbivore peut s'appuyer sur des plantes pièges attractives, des phéromones sexuelles ou des stimulants nutritionnels. Les COVs sont, quant à eux, de bons candidats pour l'une ou l'autre de ces composantes ; ils peuvent en effet repousser l'herbivore de la culture d'intérêt ou au contraire l'attirer en périphérie de la parcelle mais ils permettent aussi de recruter des ennemis naturels pour limiter les populations de ravageurs. La combinaison d'une composante « Push » et d'une composante « Pull » offre trois avantages: (i) il est possible de réduire les risques de résistances et d'adaptations développés par l'insecte en proposant à l'herbivore une situation de choix (ii) l'utilisation simultanée des deux composantes aboutit à des effets synergiques plutôt qu'additifs (iii) la concentration du ravageur dans une zone restreinte de la parcelle améliore l'efficacité de son contrôle, que ce soit à l'aide d'insecticides de synthèse dans le cadre d'une lutte intégrée ou par l'action d'ennemis naturels en lutte biologique.

### 3.2.2. L'exemple africain pour protéger les cultures de céréales : des stimuli naturellement émis

La seule stratégie de « Push-Pull » utilisée pour lutter contre des insectes ravageurs des cultures est celle mise en place en Afrique de l'Est pour protéger les cultures de maïs et de sorgho contre plusieurs lépidoptères (*e.g.* *Busseola fusca*, *Eldana saccharina*, *Chilo partellus*, *Sesamia calamistis*). Les premières recherches ont débuté au milieu des années 90 par l'observation au champ du comportement de *B. fusca* et *C. partellus* sur différentes plantes. L'une d'entre elles, l'herbe à éléphant *Pennisetum purpureum*, était nettement plus infestée que le maïs tandis qu'une légumineuse fourragère appartenant au genre *Desmodium* repoussait les ravageurs. Des études complémentaires ont ensuite montré la possibilité d'augmenter les rendements de céréales en combinant cette légumineuse en interculture (« Push ») et en déployant une ceinture d'herbe à éléphant (« Pull ») autour des parcelles (Khan *et al.*, 2000). Le rôle de *Desmodium* spp. dans le contrôle d'une herbe parasite (*Striga* spp.) a ultérieurement été mis en évidence. La relative simplicité de mise en place de cette stratégie explique son succès. Les 30000 petits agriculteurs ayant adopté ce « Push-Pull » ont pu multiplier leurs productions de maïs par trois et celles de sorgho par deux (Khan *et al.*, 2011). Au fur et à mesure de l'implantation de cette méthode, les chercheurs se sont intéressés aux mécanismes écologiques sous-jacents. Il apparaît que les odeurs émises par chacune des plantes utilisées





**Figure 4 :** L'exemple du « Push-Pull » utilisé en Afrique de l'Est

modifient le comportement des insectes à plusieurs niveaux (*cf.* figure 4). Six COVs émis par *Desmodium* spp. repoussent les ravageurs qui sont alors redirigés vers les plantes pièges. En effet, les taux d'émissions de quatre COVs attractifs, dont le (Z)-3- acétate d'hexényl, sont plus élevés chez l'herbe à éléphant que chez les céréales (Chamberlain *et al.*, 2006 ; Khan *et al.*, 2010). Il a de plus été montré que *Desmodium* spp. émet des COVs facilitant le recrutement de parasitoïdes (Midega *et al.*, 2009) et que les flavonoïdes synthétisés par cette plante sont de puissants agents allélopathiques contre les herbes parasites du genre *Striga* (Khan *et al.*, 2010).

### 3.2.3. Utilisation de COVs synthétiques

L'utilisation de COVs synthétiques dans le cadre de stratégies stimulo-dissuasives peut être bénéfique dans les deux composantes et conduire à un « Push-Pull » assisté. Il est par exemple possible de renforcer la composante « Pull » en augmentant l'efficacité de plantes pièges grâce à une odeur synthétique comme le (E)- $\beta$ -farnésène ou un mélange de trois COVs (Bennison *et al.*, 2001 ; Martel *et al.*, 2005 b). L'augmentation de l'efficacité de la composante « Pull » peut permettre de réduire sa superficie pour allouer plus d'espace à la culture d'intérêt. L'émission de COVs attirant des ennemis naturels est également intéressante pour contrôler le ravageur au sein des zones où il a été concentré (Cook *et al.*, 2007). Plusieurs études ont démontré le potentiel de COVs émis par des plantes non-hôtes dans la composante « Push ». La protection de conifères contre certains scolytes à l'aide de stratégies « Push-Pull » se base parfois sur l'émission de phéromones pour attirer le ravageur et sur la diffusion de COVs de synthèse semblables à ceux émis par des angiospermes pour le repousser (Gillette & Munson, 2007). L'utilisation d'huiles essentielles comme celle de lavande protège des navets contre le méligèthe et est envisagée pour développer une méthode « Push-Pull » adaptée à ce modèle (Mauchline *et al.*, 2013).

## 4. Contexte de la thèse, modèles biologiques et objectifs

Cette thèse s'est focalisée sur le rôle des COVs dans la structuration d'un réseau tri-trophique pour étudier la possibilité de modifier les interactions plantes-insectes au sein d'un agrosystème. Au cours de cette étude, nous nous sommes intéressés à des plantes cultivées d'importance régionale (les brassicacées) et un insecte ravageur, la mouche du chou (*Delia radicum*), causant d'importants dégâts agronomiques sur les cultures de plantes appartenant à cette famille végétale. A travers la présentation du modèle biologique sur lequel nous avons travaillé au cours de cette thèse, nous avons répertorié les différentes études s'intéressant aux rôles joués par les COVs sur le comportement de l'insecte ravageur ciblé et de ses ennemis naturels.

## 4.1. Modèles biologiques

### 4.1.1. La plante

Les cultures du genre *Brassica* occupent près de 3,4 millions d'hectares à travers le monde et constituent un marché de 26 milliards US\$ (Furlong *et al.*, 2013). Trois espèces du genre *Brassica* présentent un intérêt économique majeur : *Brassica napus* (le colza), *B. rapa* (le navet) et *B. oleracea* comprenant des variétés comme le chou-frisé (var. *acephala*), le chou-fleur (var. *botrytis*), le chou cabus (var. *capitata*), le chou de Bruxelles (var. *gemmifera*) et le brocoli (var. *italica*) (cf. figure 5). La culture de brassicacées occupe une grande importance dans l'agriculture française et européenne (cf. tableau 2). La Bretagne est la première région française de production de brassicacées légumières et concentre par exemple 73% de la production nationale de chou-fleur (DRAAF, 2008).

**Tableau 2 :** (a) Superficie des cultures de brassicacées dans l'union européenne (U.E.) et en France (2011). (b) Production de brassicacées dans l'U.E. et en France (2011). *Source : FAOSTAT (2013)*

(a)	<b>Chou-fleur et brocoli</b>	<b>Autres brassicacées</b>
<b>U.E.</b>	112 277 ha	168 043 ha
<b>France</b>	19 569 ha	4 247 ha

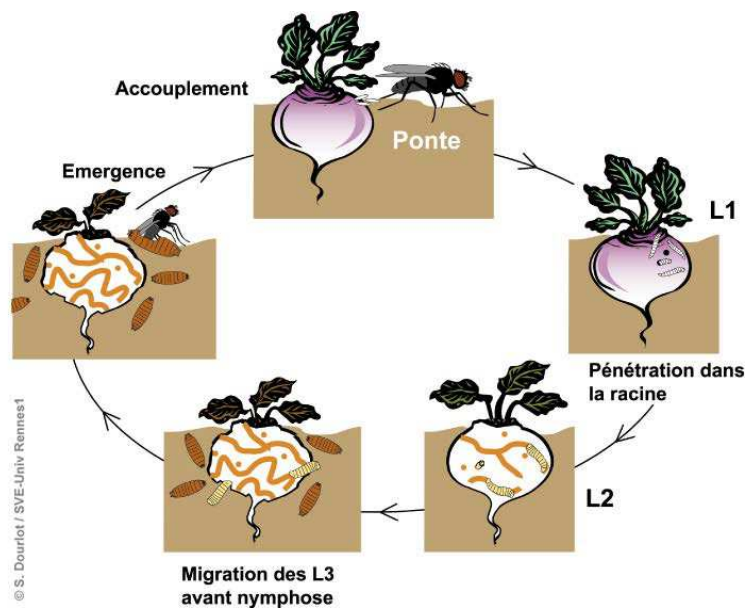
(b)	<b>Chou-fleur et brocoli</b>	<b>Autres brassicacées</b>
<b>U.E.</b>	1 952 001 tonnes	5 338 886 tonnes
<b>France</b>	364 558 tonnes	113 174 tonnes



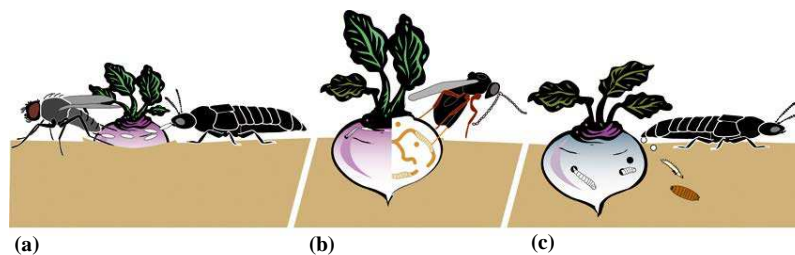
**Figure 5 :** Photo de brocoli (*Brassica oleracea* var. *italica*)

#### 4.1.2. L'herbivore et ses ennemis naturels : cycle biologique et rôle des COVs

La mouche du chou, *Delia radicum* (L. 1758) (Diptera ; Anthomyiidae) est un ravageur causant d'importants dégâts dans les cultures de brassicacées de tout l'hémisphère Nord (Biron *et al.*, 2000). Cet insecte oligophage spécialiste de la famille des Brassicaceae s'attaque par exemple aux cultures de navet, de rutabaga, de colza, de radis, de chou de Bruxelles, de choux fleurs et de brocoli (Doane & Chapman, 1962 ; Dossall *et al.*, 2000 ; Ahuja *et al.*, 2010). L'infestation d'une culture de *Brassica* par la mouche du chou entraîne une mortalité moyenne de 25% des plants et jusqu'à 90% dans des cultures non protégées (Finch, 1989). L'interdiction en 2007 du principal insecticide utilisé en France, le chlorfenvinphos et l'absence de solution chimique alternative depuis, nécessite la mise en place de nouvelles méthodes de protection des cultures. Au cours du cycle biologique de la mouche du chou (*cf.* figure 6), plusieurs ennemis naturels interviennent dans le contrôle de *D. radicum* (*cf.* figure 7).



**Figure 6** : Cycle biologique de la mouche du chou *Delia radicum* (L. 1758)



**Figure 7** : Contrôle de *Delia radicum* par les ennemis naturels à trois étapes du cycle biologique

- (a). Prédateurs des œufs
- (b). Parasitoïde larvaire
- (c). Parasitoïde des pupes



**Figure 8 :** Femelle de *Delia radicum*

**Localisation d'une plante hôte par l'herbivore** Au printemps, les adultes de *D. radicum* (cf. figure 8) émergent des pupes qui ont passé l'hiver dans le sol. Après accouplement, les femelles viennent pondre en agrégats leurs œufs au collet des plantes hôtes. Une femelle vit entre 30 et 60 jours et peut pondre jusqu'à 400 œufs (Capinera, 2001). Le rôle des bouquets d'odeurs émis par les plantes dans l'orientation des femelles à distance, a été démontré dès la fin des années 60 (Traynier, 1967 ; Finch 1978 ; Hawkes & Coaker, 1979). L'utilisation de pièges diffusant de l'isothiocyanate d'allyle permet ainsi de capturer en plein champ un grand nombre de femelles (Wallbank & Wheatley, 1979 ; Tuttle *et al.*, 1988). La distance à laquelle les stimuli olfactifs sont perçus reste cependant incertaine. Une première étude de terrain laisse penser que les COVs sont détectés à une distance de 5 à 24m de la source d'odeurs (Finch & Skinner, 1982) alors qu'une seconde étude suggère que les COVs ne sont perçus qu'au moment où la mouche du chou survole une plante hôte et ne font que stimuler son atterrissage (Finch & Collier, 2000).

**Reconnaissance d'une plante hôte par l'herbivore** Une fois que *D. radicum* est au contact d'une plante, la reconnaissance de cette plante et la décision de pondre reposent principalement sur des stimuli gustatifs comme les glucosinolates et certains dérivés de phytoalexines (Städler *et al.*, 2002). Il a cependant été montré que des stimuli olfactifs perçus

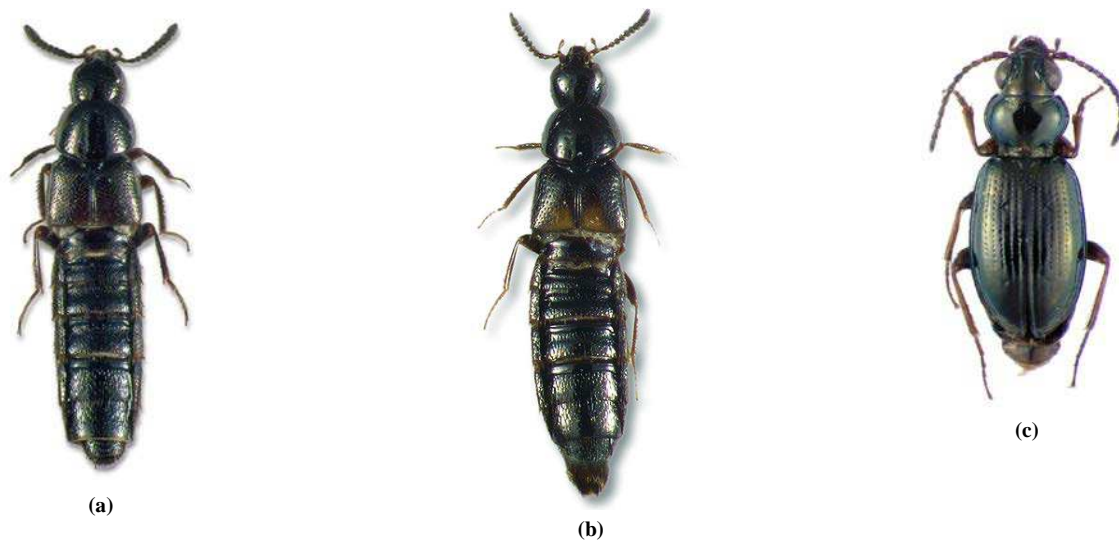


**Figure 9 :** Œufs de *Delia radicum*

après atterrissage peuvent influencer le nombre d'œufs pondus (cf. figure 9) (de Jong & Städler, 1999). Des travaux de laboratoire utilisant des femelles isolées en présence d'un site de ponte ont ainsi mis en évidence le rôle d'un COV, le disulfure de diméthyle (DMDS), dans la réduction du nombre d'œufs pondus par la mouche du chou (Ferry, 2007). Ce composé émis par des racines de brassicacées fortement infestées par des congénères est

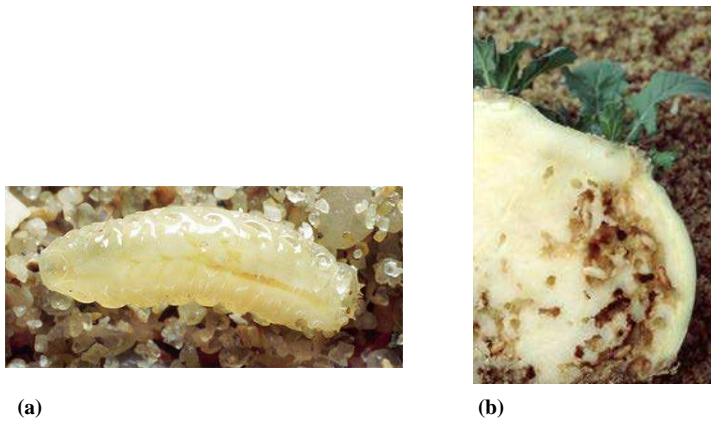
sans doute synonyme de compétition et de sites de ponte offrant peu de ressources nutritives.

**Prédateurs généralistes des œufs** Plusieurs coléoptères appartenant à la famille des Carabidae ou des Staphylinidae (*cf.* figure 10) consomment les œufs de *D. radicum* et participent au contrôle naturel de ce ravageur. En condition de laboratoire, un adulte d'*Aleochara* sp. consomme environ 60 œufs de mouche du chou par jour (Langlet, 1997). Deux études de terrains démontrent l'influence des stimuli olfactifs dans la localisation d'hôtes chez plusieurs de ces ennemis naturels que ce soit via des odeurs de plantes infestées (Goubert *et al.*, 2013) ou via des COVs de synthèse (Ferry *et al.*, 2007).

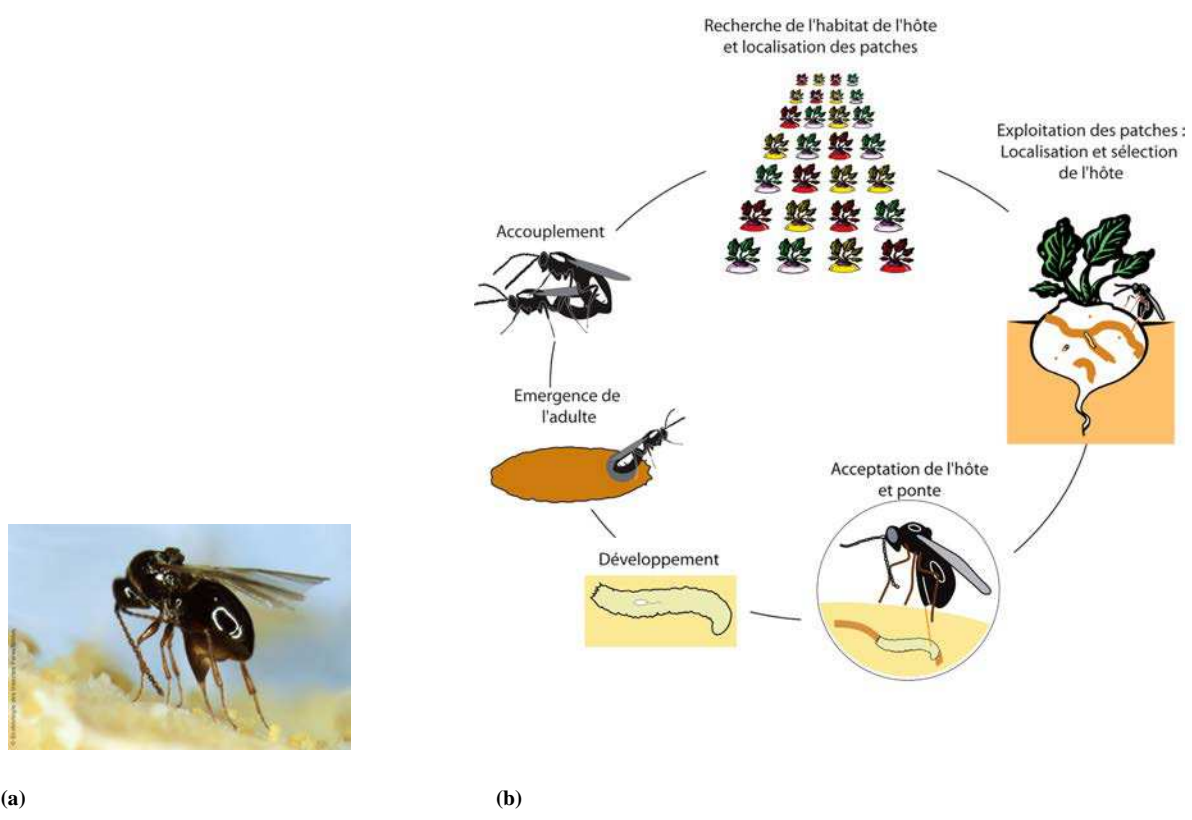


**Figure 10 :** Quelques prédateurs des œufs de *Delia radicum*  
 (a). *Aleochara bilineata* (Coleoptera : Staphylinidae)  
 (b). *Aleochara bipustulata* (Coleoptera : Staphylinidae)  
 (c). *Metallina lampros* (Coleoptera : Carabidae)

**Développement larvaire** En l'absence de prédation, les œufs pondus par la mouche du chou éclosent cinq à six jours après l'oviposition. Les larves s'enfoncent pour aller creuser des galeries dans le système racinaire où elles se nourrissent (*cf.* figure 11). Sur une période de vingt à trente jours, trois stades larvaires se succèdent en causant d'importants dégâts agronomiques. En début de saison, cinq à dix larves de mouches du chou suffisent à tuer une jeune plante (Capinera, 2001). Après trois semaines d'infestation par *D. radicum*, la biomasse racinaire peut ainsi être réduite de 47% (Blossey & Hunt-Joshi, 2003).



**Figure 11 : Développement larvaire de *Delia radicum***  
 (a). Larve isolée  
 (b). Dégâts occasionnés par des larves se nourrissant sur une racine de navet



**Figure 12 : Parasitoïde larvaire : *Trybliographa rapae* (Hymenoptera : Figitidae)**  
 (a). Femelle prospectant un site de ponte à l'aide de son ovipositeur  
 (b). Cycle de développement et localisation de plante infestées par *Delia radicum*.

**Parasitoïde larvaire** À ce stade, un hyménoptère parasitoïde, *Trybliographa rapae* (cf. figure 12), parasite les larves de *D. radicum*. Les taux de parasitisme observés sur différentes parcelles peuvent varier entre 40 et 86 % (Lahmar 1982 ; Langlet & Brunel, 1996). Les composés volatils émis par une plante infestée par la mouche du chou sont utilisés par *T. rapae* pour localiser des hôtes (Neveu *et al.*, 2002). Arrivé à proximité d'une plante infestée, le parasitoïde profite des anfractuosités du sol autour des racines pour pénétrer dans une galerie creusée par une larve de la mouche du chou avant de venir pondre ses œufs à l'intérieur de celle-ci.

**Nymphose** Parasitées ou non par *T. rapae*, les larves de *D. radicum* s'éloignent de la racine une fois qu'elles se sont suffisamment nourries et vont se nymphoser dans le sol. En fonction du génotype de la plante hôte et des



Figure 13 : Pupa de *Delia radicum*

performances larvaires, entre 45% et 78% des larves forment une pupa (cf. figure 13) (Hopkins *et al.*, 1999).

**Parasitoïdes des pupes** Les pupes de *D. radicum* peuvent être parasitées par les larves d'*A. bipustulata* et d'*A. bilineata*. En effet, alors que les adultes de ces deux espèces de staphylins consomment les œufs de la mouche du chou, *A. bipustulata* et *A. bilineata* font également partie des rares coléoptères à présenter un statut de parasitoïde (cf. figure 14). En plein champ, le taux de parasitisme des pupes de la mouche du chou par les larves d'*Aleochara* sp. varie autour d'une moyenne estimée à 20% (Fournet, 2000 ; Bonsall *et al.*, 2004).

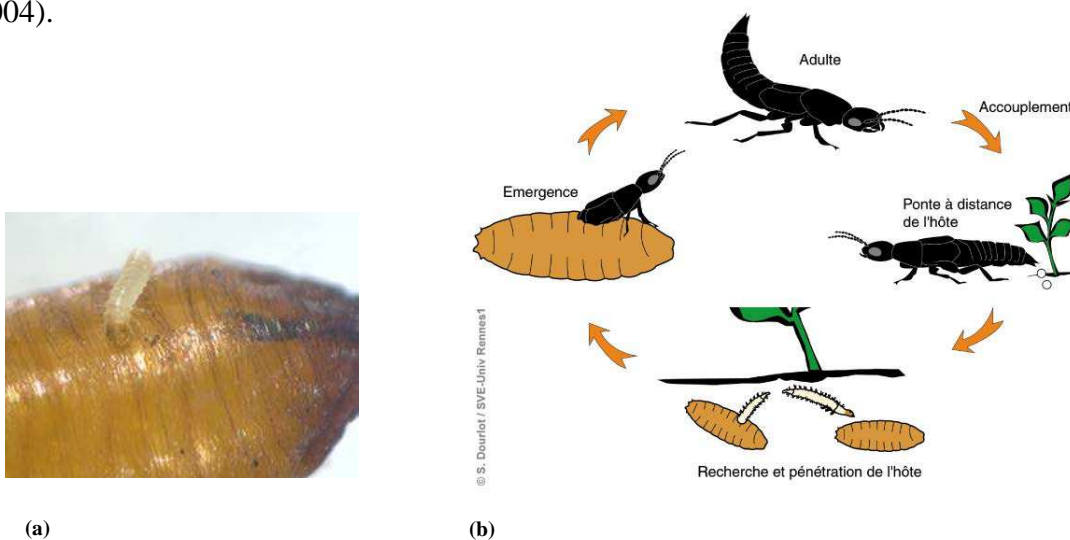


Figure 14 : Parasitoïde des pupes : *Aleochara bilineata* et *A. bipustulata* (Coleoptera : Staphylinidae)

(a). Larve d'*Aleochara* sp. cherchant à pénétrer une pupa de mouche du chou  
 (b). Cycle de développement



**Emergences** À partir du stade de pupes, il faudra une vingtaine de jour à une mouche pour émerger, une trentaine de jour pour un adulte d'*Aleochara* sp. et une soixantaine de jour pour un adulte de *T. rapae*. Sur le terrain, il est possible d'observer sur une même saison trois générations chevauchantes de mouches du chou. Un premier pic d'émergence a lieu entre les mois de mars, avril et mai. Les pupes du dernier pic entrent en diapause et passent l'hiver dans le sol. Elles n'émergeront qu'après vingt-deux semaines passées en dessous de 6°C (Capinera, 2001).

## 4.2. Objectifs de la thèse et présentation des articles

Les odeurs de plantes jouent un rôle à plusieurs niveaux au sein du système tri-trophique étudié, que ce soit sur le comportement de l'herbivore *D. radicum* ou sur celui de ses ennemis naturels, orientant notamment leurs choix d'une ressource trophique ou d'un substrat de ponte. Cependant, très peu de stimuli olfactifs ont été identifiés jusqu'à maintenant et leurs intérêts en tant que leviers comportementaux dans un contexte agronomique nécessite de vérifier leurs potentiels en plein champ avant d'étudier leur déploiement à l'intérieur de parcelles. Le travail effectué au cours de cette thèse a donc deux objectifs principaux :

- (i) explorer le registre de stimuli olfactifs impliqués dans la structuration du système tri-trophique brassicacées – mouche du chou – ennemis naturels. De telles substances sémiochimiques pourraient servir à la mise en place d'une méthode de protection des cultures basée sur la modification comportementale du deuxième et/ou du troisième niveau trophique.
- (ii) étudier la mise en place de leviers comportementaux au sein de parcelles cultivées. Dans le cadre d'une stratégie stimulo-dissuasive, des stimuli négatifs pourraient permettre le développement d'une composante « push » alors que des stimuli positifs seraient utiles à la composante « pull ».

Nous avons été amenés au cours de cette thèse à développer une démarche générale combinant des études en laboratoire et des études en plein champ. Afin d'identifier des plantes intéressantes dans le développement des composantes « push » et « pull », l'observation du comportement de l'herbivore en plein champ permet de prendre en compte des paramètres environnementaux (*e.g.* qualité de la plante face aux conditions climatiques ou aux autres herbivores présents) pouvant déterminer le succès d'une stratégie de lutte contre le

ravageur ciblé. Cependant, en incluant de nombreux facteurs non contrôlés ce type d'expérimentation rend difficile l'identification de stimuli responsables du comportement observé. Les plantes sélectionnées sur le terrain en fonction de leurs degrés d'infestation ont donc fait l'objet d'expérimentations en laboratoire ayant pour but de déterminer à l'aide d'études olfactométriques l'influence des odeurs végétales sur le comportement de *D. radicum*. Une analyse en chromatographie gazeuse à partir des profils de COVs émis par les plantes ainsi sélectionnées nous a permis d'identifier des composés volatils potentiellement impliqués dans les prises de décisions de la mouche du chou. Alors que la grande majorité des études portant sur les interactions plantes – insectes sont réalisées au laboratoire, nous avons essayé de modifier au sein de cultures le comportement du ravageur et/ou de ses ennemis naturels à l'aide de différentes espèces végétales ou de COVs synthétiques. Dans un contexte appliqué, des études de plein champ permettent d'étudier le comportement des insectes au sein de leurs réseaux trophiques complets et donc de prendre en compte des effets non ciblés (e.g. répulsion des ennemis naturels et altération des effets « top-down ») qui sont difficiles à prédire au laboratoire. Cette démarche générale permet de présenter la thèse sous la forme de cinq articles en cours de préparation ou déjà publiés.

**Article 1.** L'objectif de ce premier article est double. Nous souhaitons tout d'abord identifier des plantes permettant la mise en place d'une stratégie « Push-Pull » à l'aide de variétés de brassicacées. Une étude menée en plein champ a permis de sélectionner des plantes présentant différents degrés d'infestation vis-à-vis de la mouche du chou. En combinant cette première sélection de plantes avec des observations comportementales en olfactométrie nous voulons ensuite identifier des COVs impliqués dans les prises de décisions de *D. radicum*.

**Article 2.** Dans un deuxième temps, nous avons élargi le nombre d'observations olfactométriques pour (i) confirmer le rôle des COVs dans les prises de décisions de *D. radicum* (ii) obtenir des plantes contrastées vis-à-vis de leur attractivité. Ces observations comportementales ont été associées à une analyse en chromatographie gazeuse des profils de COVs émis par les différentes plantes testées. Nous espérons sélectionner au sein des bouquets d'odeurs d'origine végétale des substances sémiochimiques pouvant être intéressantes dans le cadre d'un « Push-Pull » assisté.

**Article 3.** L'objectif de cette expérimentation de terrain est de vérifier le potentiel des COVs dans un contexte agronomique. Nous avons donc mis en place des diffuseurs d'odeurs

synthétiques au sein d'une parcelle. Les résultats montrent qu'il est possible de modifier localement le comportement de ponte de la mouche du chou et l'activité de ses ennemis naturels à l'aide de stimuli olfactifs.

**Article 4.** Les résultats précédents étant encourageants, nous avons étudié le moyen de déployer efficacement des diffuseurs de COVs synthétiques à l'intérieur d'une parcelle. Pour cela, nous avons observé l'influence des diffuseurs à différentes distances de la source d'odeurs synthétique sur (i) le comportement de ponte de la mouche du chou (ii) le nombre de pupes retrouvées en fin de saison et (iii) les taux d'émergences de la mouche du chou et de ses parasitoïdes.

**Article 5.** En parallèle, nous avons également étudié l'infestation de plants de brocoli entourés par trois ceintures végétales différentes pour estimer leurs potentiels dans une stratégie « Push-Pull ». Parmi les trois ceintures testées, nous avons sélectionné deux espèces végétales préalablement décrites comme pouvant représenter des plantes pièges efficaces et une espèce végétale présentant des faibles taux d'infestations. Nous espérons ainsi comparer l'efficacité de ceintures « Pull » et « Push » dans la protection des cultures de brassicacées contre *Delia radicum*.

Ce manuscrit se termine par une discussion générale reprenant les principaux résultats obtenus dans chacun des cinq articles pour les mettre en perspectives les uns avec les autres. La discussion générale sera également l'occasion d'ouvrir la réflexion sur les prochaines étapes à mettre en place pour compléter le travail initié dans cette thèse avant de penser à développer une stratégie « Push-Pull » combinant des ceintures végétales et des COVs de synthèse.

**ARTICLE N°1 : Sélection en plein champ de plantes modulant la ponte de *Delia radicum* pour développer une stratégie “Push-Pull” contre ce ravageur**

*Article en préparation*



# Field selection of brassicaceous plants that differentially affect oviposition levels by the cabbage root fly and could be used in a ‘push-pull’ strategy.

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## ABSTRACT

Several plant traits control plant-insect interactions and shape host range of herbivorous insects according to their degree of dietary specialization. Understanding how host-plant diversity influences herbivore infestations is of interest for the development of alternative crop protection strategies. In a pest management context, an appropriate selection of plants can modify pest repartition at the field scale. In order to develop a “push-pull” strategy against the cabbage root fly, *Delia radicum*, we conducted a field study to select plants exhibiting contrasted pest infestation levels and evaluate their influence on egg-predation activity. Our field experiment reveals that infestation levels of brassicaceous plants by the cabbage root fly in the field can vary considerably according to plant genotype and species, while number of predated eggs is only slightly affected by plant species. Olfactometry studies carried out in laboratory conditions revealed that plants harboring the highest number of eggs in the field were also the most attractive ones, suggesting that olfactory stimuli influence the differential infestation levels observed in the field. In a “push-pull” context, the present study demonstrates the possibility to select plants that could be used to redistribute cabbage root flies in broccoli crops without compromising herbivore control by natural enemies. In addition, the importance of plant volatiles in infestation levels suggests a potential for developing a semiochemically assisted ‘push-pull’ where trap plants would be enhanced by synthetic release of attractive VOCs.

**Key words:** Plant preference, cabbage root fly, egg predator, infestation level, attractiveness, olfactory stimuli.

# INTRODUCTION

Several plant traits, such as chemistry, physiology or morphology, control plant-insect interactions and shape host range of herbivorous insects according to their degree of dietary specialization (Schoonhoven *et al.*, 2005). Even insects specialized on a particular plant family, such as the Brassicaceae, display different degrees of preference at both inter- and intra-specific levels (Griffiths *et al.*, 2001; Städler *et al.*, 2002; Poelman *et al.*, 2009). Understanding how host-plant diversity influences herbivore infestations is of interest not only from an ecological point of view but also for the development of alternative crop protection strategies (Ratnadass *et al.*, 2012). All pests show distinct preferences for particular plant species, cultivars or growth stages. In a pest management context, an appropriate selection of plants can modify pest repartition at the field scale. The ‘push-pull’ strategy uses plant diversification to combine a commercial crop with both repellent plants (‘push’) in inter-culture and trap plants (‘pull’) surrounding the field (Hokkanen, 1991; Shleton & Badenes-Perez, 2006; Cook *et al.*, 2007). This strategy aims at reducing pest density in the commercial crop while concentrating its populations in limited areas where they can be controlled.

The ‘push-pull’ strategy relies essentially on antixenotic and antibiotic resistances of plants. Antixenosis is the ability of a plant to repel or deter insects, causing a reduction in oviposition or feeding. Volatile organic compounds (VOCs) released by plants play an important role in antixenotic resistance of plants (Schoonhoven *et al.*, 2005; Smith *et al.*, 2005). These volatiles convey different information to herbivores such as host plant location, plant phenology and presence of both competitors and natural enemies (Visser, 1986; Sabelis *et al.*, 2001; Szendrei *et al.*, 2009; Bruce & Pickett, 2011) and thereby greatly influence crop colonization. Antibiosis is the ability of a plant to reduce the survival, growth, or reproduction of insects that feed on it. The selection of plants simultaneously exhibiting low degrees of antixenosis and high degrees of antibiosis can lead to ‘dead-end’ trap cropping. In this case trap crops can be transformed in pest sinks where dissemination is limited in either space (from the trap to the commercial field) or time (over the seasons) (Shleton & Badenes-Perez, 2006). In a multi-trophic context, herbivore control through antibiotic mechanisms (bottom-up control) can be reinforced by natural enemies (top-down control). Host foraging by predators also often involves plant VOCs (Dickens, 1999; Dicke & Baldwin, 2010; Hare, 2011). Therefore, the selection of a plant for developing a ‘push-pull’ strategy should also focus on the influence of host plant diversity on pest attack rate by natural enemies.

The cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae), is a worldwide pest specialized in brassicaceous plants and reducing yields in many crops such as cauliflower, turnip, rutabaga and broccoli (Finch, 1989; Meyling *et al.*, 2013). Western Europe is an important area of brassica production where broccoli is usually transplanted in the field early in the season (March, April) thereby being exposed to winter emerging generations of the cabbage root fly at a highly vulnerable stage. The recent limitation of insecticides toward *D. radicum* in Europe requires the development of alternative crop protection methods and behavioural manipulation appears like a promising approach. *Delia radicum* females lay eggs in the soil close to plant stem and larva cause agronomic damages by feeding on the roots. Ground dwelling egg predators including carabidae and staphylinidae are particularly important for natural control of the fly (Coaker & Williams, 1963; Prasad & Snyder, 2004). The host plant finding behaviour of the cabbage root fly is divided in different sequences involving various stimuli although their relative importance has been debated (Finch & Collier, 2000). At a distance ranging from 5m to 24m, VOCs are involved in cabbage root fly attraction (Finch & Skinner, 1982). The landing sequence is then mediated by visual cues (Roessingh & Städler, 1990). Finally, host plant acceptance relies essentially on chemocontact stimuli (Städler *et al.*, 2002) although synergistic effects between plant odours and on-ground stimuli have been suggested (de Jong & Städler, 1999). Due to the variety of plant stimuli involved in host plant recognition, plant diversity could strongly affect cabbage root fly behaviour.

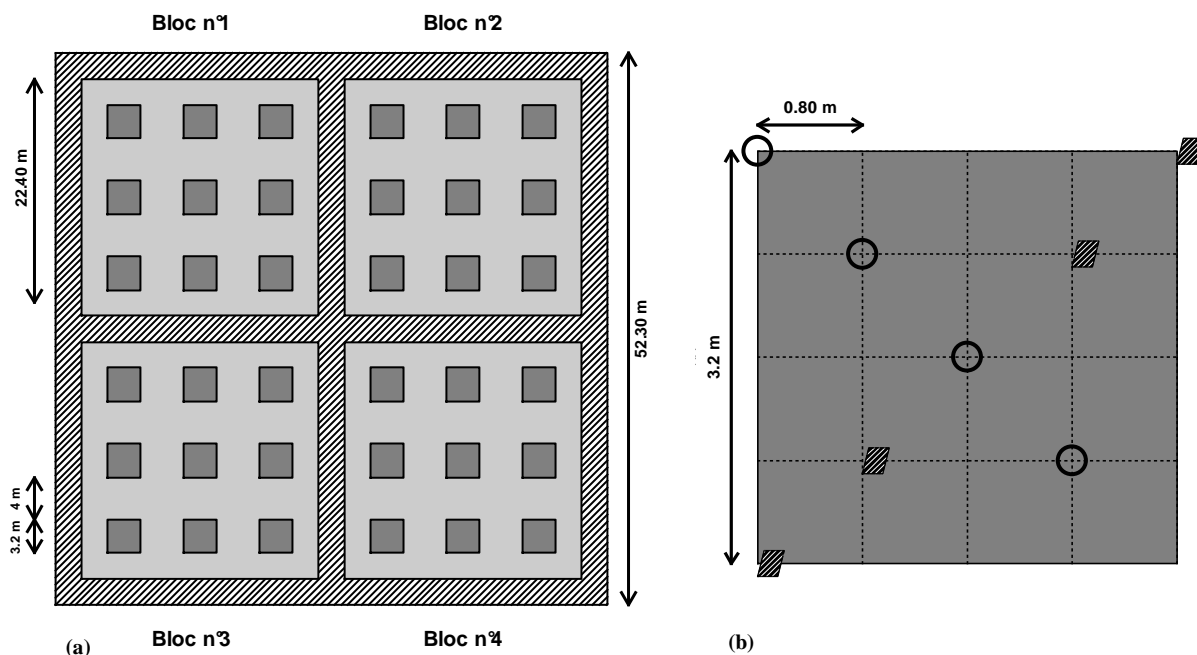
The objective of the present study is to identify, under field conditions, brassicaceous plants which differentially affect infestation by the cabbage root fly and can later be used in a ‘push-pull’ approach to protect broccoli (*Brassica oleraceae*) crops against this pest. We assessed oviposition of *D. radicum* on plants that were previously described as resistant to the cabbage root fly, *Sinapis alba* and *B. juncea* or particularly susceptible, *B. rapa* (Doddall *et al.*, 1994). Two genotypes of oilseed rape, *B. napus* ‘Yudal’ and ‘Darmor-bzh’, were also retained due to the variability of susceptibility within *B. napus* species (Doddall *et al.*, 2000) and the contrasted responses of these two plants to different pathogens (Manzanares-Dauleux *et al.*, 2000; Delourme *et al.*, 2008). In addition to the assessment of cabbage root fly oviposition, we monitored egg predation levels in plots of these different plants. Furthermore, we investigated whether or not VOCs played an important part in the differential infestation levels of these plants by the pest through olfactometer tests conducted in laboratory conditions.



# MATERIALS AND METHODS

## Field experiment

**Field setup** The field experiment was performed during spring 2011 in broccoli fields (*Brassica oleraceae* L. var. *Italica* cv. 'Marathon') located at the experimental station of "La Motte" (INRA Center), Le Rheu, Brittany, France (48°06'36" N, 1°48'05" W). Broccoli seeds were sown individually in peat soil cylinders and grown for 6 weeks in a plastic tunnel (commercial provider: Thomas Plants, Ploubazlanec, France). The soil was fertilized at 63.5 kg nitrogen per ha before plantation. One week later, the broccoli seedlings were transplanted in the field (6 and 7 April). The field was cultivated according to usual agricultural practices and no pesticides were applied. We used a randomized block design consisting of 4 blocks (29x29 plants, 502m<sup>2</sup>) with 9 experimental plots (5x5 plants, 10.2m<sup>2</sup>) in each block (Fig. 15) Blocks were separated from each other by 2.5m of bare soil while plots in the blocks were separated by broccoli strips of 4m. In all plots except controls, broccoli was replaced on the 7<sup>th</sup> and 8<sup>th</sup> of April with the different plants tested.



**Figure 15 :** Experimental field setup

(a) Four blocks (light grey) of broccoli, *Brassica oleraceae* var. *Italica* cv. 'Marathon', were planted in bare soil (hatching). Each block contained 9 randomized plots (dark grey) of different plants. One control plot was planted with broccoli and the five other plots kept for the analysis were planted with Indian mustard (*B. juncea*), turnip (*B. rapa*), two genotypes of oilseed rape (*B. napus*, 'Yudal' and 'Darmor-bzh' genotypes) and white mustard (*Sinapis alba*).

(b) Each plot consisted of a square of 5x5 plants. Oviposition of *Delia radicum* was monitored with felt traps (open circle) placed on 4 different plants while predator activity was estimated with artificial patches of cabbage root fly eggs (hatched diamond) placed on the soil next to 4 other plants.

**Genotypes tested** Eight plant genotypes were tested: two genotypes of oilseed rape (*B. napus* - ‘Yudal’ and ‘Darmor-bzh’ genotypes), two genotypes of turnip (*B. rapa* cv. ‘Nancy’ and cv. ‘Chicon’), two genotypes of broccoli (*B. oleraceae* cv. ‘Hdem’ and cv. ‘CB-151’) and two mustard species (*B. juncea* and *Sinapis alba*). Three plant genotypes were drastically affected by drought and were excluded from the analyses (*B. oleraceae* cv. ‘Hdem’ and cv. ‘CB-151’, *B. rapa* cv. ‘Chicon’). Hereafter, *B. oleraceae* refers to the ‘Marathon’ cultivar while *B. rapa* refers to the cultivar ‘Nancy’. Plants tested were sown on the 8<sup>th</sup> of February in compost soil cylinders (60cm<sup>3</sup>, blond peat/vermiculite/perlite), were grown in a greenhouse (14h:10h L:D, 20°C:17°C L:D) and were watered twice a week with a nutrient solution (N:P:K:Mg 2.5:5:2.5:0.75 and oligo-elements). Plants with 3-4 fully developed leaves were stored in a cold chamber (8h:16h L:D, 5±1°C) until plantation.

**Cabbage root fly oviposition** Egg laying by *D. radicum* was monitored using felt traps (for a description, see *Bligaard et al.*, 1999) positioned around the stem of the plants, where flies deposit their eggs. Felt traps were placed on four plants in each plot (Fig. 15). Traps were collected every week and the eggs found inside were counted and removed. Emptied traps were then replaced on the same plants (or on the nearest plant when this plant had died). Cabbage root fly infestation was monitored from April 12 to May 10.

**Egg predation** Egg predation in the plots was assessed using sentinel patches of eggs. These patches consisted of a 1 cm<sup>2</sup> piece of black paper pinned down to the soil surface and protected from the rain using a small plastic cover. Fifteen *D. radicum* eggs collected in our rearing facility were glued onto the paper with gum arabic. Four patches were placed next to the stem of four plants in each elementary plot and maintained in the plots for 48 h, after which the number of predated egg was recorded. This experiment was repeated three different weeks (27 April, 11 and 20 May). The presence of predators (*Aleochara* spp. and Carabidae) in the experimental field was assessed using two pitfall traps placed in each block and monitored weekly. Predation tests were carried out when more than 15 adults were found per trap.

## Olfactometer bioassays

**Plants** Three genotypes of plants exhibiting contrasted levels of infestation toward *D. radicum* in the field were retained for olfactometer bioassays: *Brassica oleracea* cv.

'Marathon', *B. napus* 'Yudal' genotype and *B. napus* 'Darmor-bzh' genotype. Plants were grown as previously described and after three weeks, were transplanted in 9x9x10 cm pots filled with compost soil (blond peat/ black peat/ perlite) and watered twice a week with a nutrient solution (N:P:K:Mg 2.5:5:2.5:0.75 and oligo-elements). Plants used for experiments were five weeks old corresponding to 6-8 leaves.

**Insects** Females of *Delia radicum* used for the experiments originated from a colony started in July 2012 from field collected flies (Le Rheu, Brittany, France, 48° 07' 16'' N, 01° 47' 41'' W). The fly colony was fed with a milk powder:yeast:sugar (1:1:1) mixture and kept in a climatic chamber at 16h:8h L:D, 20±2°C and 55±5% RH. The rearing procedure was adapted from Neveu Bernard-Griffiths, 1998. Three days after emergence, females were considered as mated and were used for experiments until they were 10 days old. All tests were conducted in a lighted room maintained at 20±2°C and 60±10% RH. Flies tested were isolated and placed in the test room at least 15 min before assays for acclimatation.

**Behavioral tests** Behavioural responses of *D. radicum* to control (*i.e.* pure air) or odours emitted by the three plants tested were monitored using a tubular olfactometer (60cm x 5cm ID) artificially divided into six notional sections of 10 cm. Light was supplied by one neon light (36W) placed above the tube. The airflow was provided with a bottle of synthetic air (N:0 / 80:20) moistened before reaching a plant enclosed in a PET oven bag (polyethylene terephthalate). Airflow in the olfactometer was maintained at 400ml.min<sup>-1</sup>. All connections were made with PTFE tubing (polytetrafluorethylene). Females were placed individually in the tube and their behaviour was recorded during 10 minutes. The tubular olfactometer was placed in a white box in order to avoid any visual stimuli from the plant.

For each treatment 3 or 4 distinct plants were used and 28 to 30 females were tested (between 4 and 12 per plant). Olfactometer tests were carried out during 4 weeks (November 2012). Treatments (including control) were randomized during this period. The entire device was washed with ethanol, dried and purged during fifteen minutes with clean air before starting new observations.

## Statistical analysis

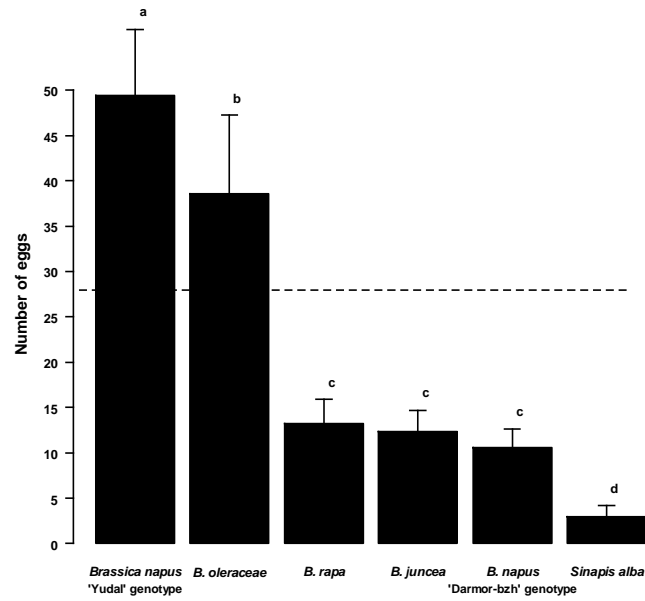
Statistical tests were performed with R software, version 2.12.1 (R Development Core Team, 2011). Analyses about cabbage root fly oviposition in the field experiment were conducted on the cumulative number of eggs laid per plant over the four sampling weeks. Cabbage root fly oviposition and number of eggs predated in the artificial patches were analyzed through generalized linear mixed models (GLMM, package 'lme4') for Poisson data (linking function: 'log'). One random factor ('block') was retained for the model assessing the cabbage root fly oviposition while two random factors were included in the model about egg predation ('block' and 'sampling date'). For both, GLMM, comparisons between treatments were carried out using analyses of contrast (package 'doBy') and p.values were adjusted by a false discovery rate correction method.

The package 'TraMineR' was used to visualize behavioural sequences obtained in olfactometer tests (Gabadinho *et al.*, 2011). Mean time (s) spent in each section of the olfactometer was assessed with a generalized linear model (GLM, package 'lme4'). The goodness-of-fit of each model tested was estimated by graphical observations of (i) the model residuals plotted against the fitted values (ii) the quantile randomized residuals plotted against the normal distribution quantiles. A GLM with 'quasipoisson' distribution (linking function: 'log') was finally retained to take account of over-dispersion. Mean time differences were estimated by contrasts methods applied to GLM (package 'doBy') and p.values were adjusted by a false discovery rate correction method.

## RESULTS

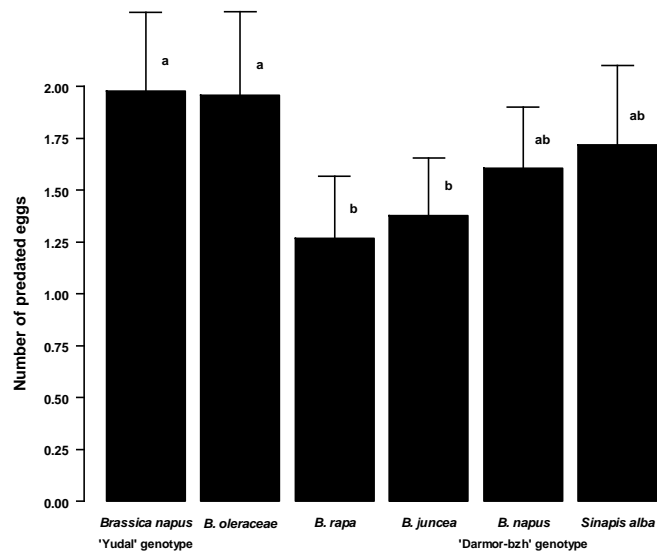
### Field experiment

**Cabbage root fly oviposition**      Number of eggs laid by *D. radicum* varied according to the plant tested (GLMM for Poisson data, likelihood ratio test:  $\chi^2 = 933.42$ , 5 d.f.,  $P < 0.001$ ). Over the four sampling weeks, the infestation level of *Brassica napus* 'Yudal' genotype and *B. oleraceae* exceeded the threshold of 7 eggs.week<sup>-1</sup>.plant<sup>-1</sup> used by farmers to decide whether or not to spray against the pest (Fig. 16). One oilseed rape genotype (*B. napus* 'Yudal' genotype) was more infested by *D. radicum* than broccoli while the four other plant genotypes harboured fewer eggs.



**Figure 16 :** Mean ( $\pm$  SE) cumulative number of *Delia radicum* eggs

Number of eggs found per felt trap was cumulated on four sampling dates (19 and 26 April, 3 and 10 May). The dashed line corresponds to the threshold of 28 eggs per felt trap per month, which is habitually used by farmers to decide whether or not to spray against the pest. Means with different letters are significantly different: GLMM 'poisson', analysis of contrast and P.value correction (false discovery rate),  $P < 0.05$



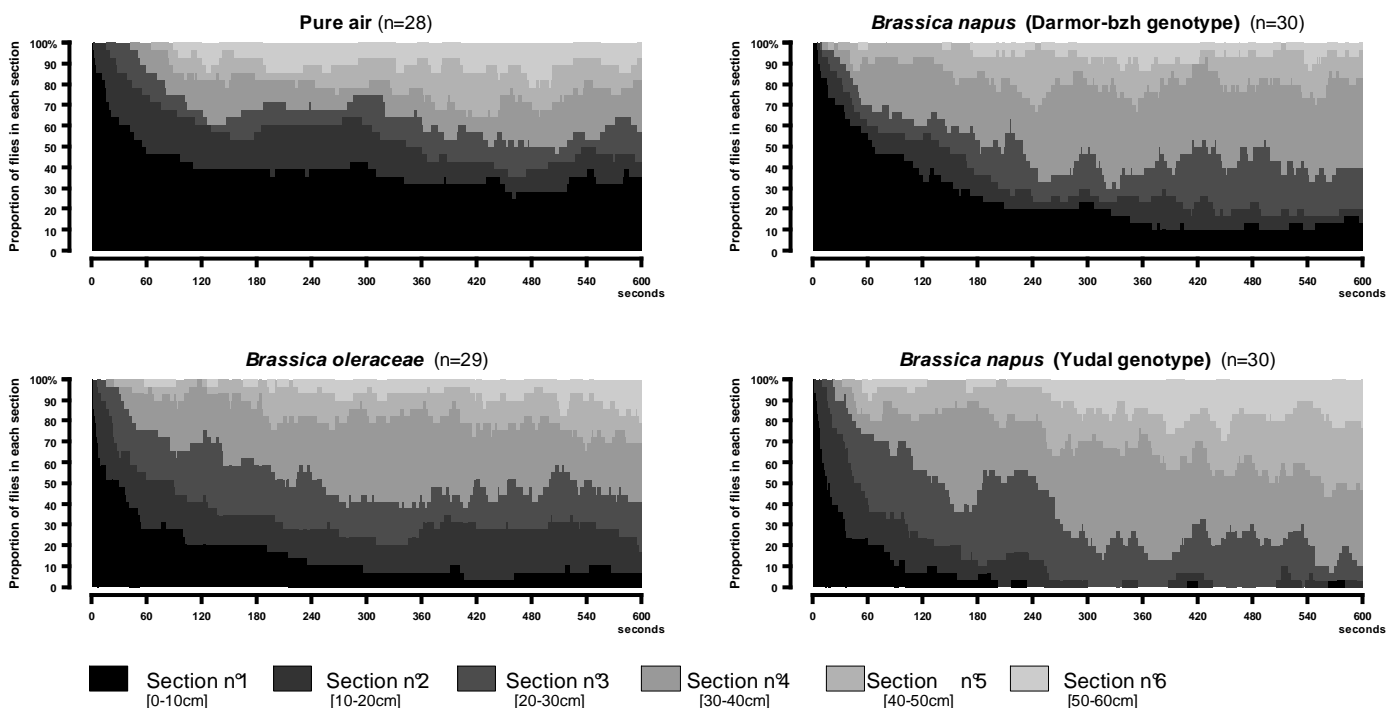
**Figure 17 :** Mean ( $\pm$  SE) number of predated *Delia radicum* eggs

The mean number of predated eggs was assessed on artificial egg patches exposed for 48h to predators. Experiments were repeated three times (27 April, 11 and 20 May). Means with different letters are significantly different: GLMM 'poisson', analysis of contrast and P.value correction (false discovery rate),  $P < 0.05$

**Egg predation** Among the 268 artificial eggs patches tested over the three weeks of predation experiments, the proportion of patches with predated eggs (*i.e.* visited by predators) fluctuated between 42% (*B. rapa*) and 60% (*B. napus* 'Darmor-bzh' genotype) but was not significantly different among plant genotypes ( $\chi^2 = 3.60$ , 5 d.f.,  $P = 0.61$ ). The mean number of predated eggs per patches differed slightly with treatments (GLMM for Poisson data, likelihood ratio test:  $\chi^2 = 12.21$ , 5 d.f.,  $P = 0.032$ ). The number of predated eggs in both turnip and Indian mustard plots was lower than in broccoli plots (Fig. 17).

## Olfactometer bioassays

The time spent in the first section of the olfactometer and the patterns of movements of flies in the other sections provide interesting information about behavioural responses. While the time spent in the first section corresponds to latency or absence of response, the pattern of movements in the other sections reflects plant attractiveness. In a control airflow, *i.e.* without any plant odour, about 30% of flies stayed in the first section of the olfactometer over the ten minutes of recording (Fig. 18). This proportion decreased to 10 % (*B. napus*



**Figure 18** : Sequential responses of *D. radicum* females exposed to various brassicaceous plants and to pure air in a tubular olfactometer artificially divided in 6 notional sections (section n°1: fly entrance; section n°6: entrance of airflow in the tube)

Movements between sections were recorded during ten minutes (600 seconds)

'Darmor-bzh' genotype) or less than 10% (*B. oleraceae* and *B. napus* 'Yudal' genotype) when flies were exposed to plant volatiles. The mean time spent in the first section of the olfactometer was higher than in the last section of the olfactometer when flies were exposed to pure air and *B. napus* 'Darmor-bzh' while they were similar with plant volatiles released by broccoli and the 'Yudal' oilseed rape genotype indicating a higher attractivity (Table 3). However, two different patterns of movements can be distinguished for the broccoli and the 'Yudal' genotype. Flies exposed to broccoli volatiles spent as much time in sections 3-4 of the olfactometer as in sections 1-2 while flies exposed to 'Yudal' spent more time in sections 3-4 suggesting that it is the most attractive plant tested.

**Tableau 3 :** Mean time in second ( $\pm$  SE) spent by *D. radicum* females exposed to various brassicaceous plants and to pure air in a tubular olfactometer artificially divided in 6 notional sections (section n°1: fly entrance; section n°6: entrance of airflow in the tube)

	Pure air	<i>Brassica napus</i> 'Darmor-bzh' genotype	<i>Brassica oleracea</i>	<i>Brassica napus</i> 'Yudal' genotype
<b>section n°1</b> [0-10 cm]	236 $\pm$ 46 a A	154 $\pm$ 31 ab AB	93 $\pm$ 27 ab BC	39 $\pm$ 8 a C
<b>section n°2</b> [10-20 cm]	100 $\pm$ 26 b AB	57 $\pm$ 14 cd A	117 $\pm$ 29 ab B	54 $\pm$ 10 a A
<b>section n°3</b> [20-30 cm]	64 $\pm$ 16 b A	97 $\pm$ 15 ac AB	120 $\pm$ 24 ab AB	146 $\pm$ 21 bc B
<b>section n°4</b> [30-40 cm]	82 $\pm$ 23 b A	193 $\pm$ 25 b B	172 $\pm$ 27 b B	195 $\pm$ 22 b B
<b>section n°5</b> [40-50 cm]	64 $\pm$ 20 b NS	72 $\pm$ 11 c NS	62 $\pm$ 14 ac NS	104 $\pm$ 15 cd NS
<b>section n°6</b> [50-60 cm]	54 $\pm$ 22 b NS	28 $\pm$ 9 d NS	35 $\pm$ 14 c NS	63 $\pm$ 17 ad NS

Mean times were calculated after ten minute (600 s) of observation. Significant differences in mean times are represented with different letters: GLM 'quasipoisson', contrasts method and P.value correction (false discovery rate)  $P < 0.05$ . Minuscule letters compare mean times spent in each section for a same treatment while capital letters compare mean times between treatments for a same section of the olfactometer

## DISCUSSION

Our field experiment confirms that infestation levels of brassicaceous plants by the cabbage root fly in the field can vary considerably according to plant genotype and species. Over the four weeks of monitoring, number of eggs laid on the 'Yudal' oilseed rape genotype was tenfold higher than on white mustard *S. alba*. Plants harboring the highest number of eggs in the field were also the most attractive ones in the olfactometer. Confronting field experiment and behavioral observations in the olfactometer suggests that olfactory stimuli may influence the infestation rates in the field.

Relatively to broccoli (*B. oleraceae*), egg laying of *D. radicum* was reduced on four plants (*S. alba*, *B. napus* ‘Darmor-bzh’ genotype, *B. juncea* and *B. rapa*) while one plant (*B. napus* ‘Yudal’ genotype) increased oviposition. The high degree of infestation on broccoli fits well with the susceptibility of this crop towards the cabbage root fly. An intermediate level of infestation for *B. juncea* was previously recorded under field conditions and Dossall *et al.*, 1994 recommended to canola growers to seed it rather than *B. rapa* in regions where high damages of the cabbage root fly were observed. The white mustard *S. alba* presents strong resistant traits through both antixenosis and antibiosis and has been used in plant breeding to confer protection to *B. napus* against the cabbage root fly (Dossall *et al.*, 1994; Ekuere *et al.*, 2005). In addition to previous studies demonstrating the role of chemocontact stimuli in oviposition choices in *D. radicum* (Städler *et al.*, 2002), physical factors and morphological traits (*e.g.* surface cover, colours, and tissue tenderness) can also influence oviposition behaviour (Hardman & Ellis, 1978; Roessingh & Städler, 1990). The white mustard *S. alba* is the only plant tested to harbour trichomes on the stem and it has been suggested that such physical barriers may reduce egg laying of the cabbage root fly (Jyoti *et al.*, 2001). Although we cannot exclude that volatiles released by *S. alba* also play a role, this plant was not retained for olfactometer bioassays due to the probable influence of physical stimuli such as trichomes. One genotype of oilseed rape (*B. napus* ‘Darmor-bzh’), harboured few eggs while the other, (‘Yudal’), was the one where the highest numbers of eggs were found among all the plants tested. The ‘Darmor-bzh’ genotype is a dwarf mutant (obtained at INRA, Rennes by Foisset *et al.*, 1995) with a shorter stem than the ‘Yudal’ genotype. After landing on a host-plant, the oviposition of *D. radicum* is preceded by a complex behaviour pattern involving different sequences with two phases occurring on the stem (Zohren, 1968 cited by Schoonhoven *et al.*, 2005). The informational uptake by the cabbage root fly could be disrupted on dwarf mutants with short stems resulting in a reduction of the number of eggs laid (Roessingh & Städler, 1990). Also, volatiles emitted by the two genotypes seem to play an important role as suggested by our olfactometric tests (see below). In the present study, turnip (*B. rapa*) presents a low infestation level although it has been shown to be susceptible to the cabbage root fly in previous studies (Doane & Chapman, 1962; Dossall *et al.*, 2000). It has even been proposed as a possible trap crop to protect broccoli crops against *D. radicum* (Rousse *et al.*, 2003). In our field study, we observed a strikingly high attack rate of several herbivores such as *Pieris* spp. (Lepidoptera: Pieridae) and flea beetles (Coleoptera: Chrysomelidae) on the turnip cultivar tested compared to the other plants tested. This high



level of infestation by other herbivores may have altered the quality of the plant for the cabbage root fly and could be responsible for the low infestation levels observed.

The influence of the different plants tested on egg predator activity seems to be relatively weak in comparison to its influence on the cabbage root fly behaviour. No differences in the proportion of patches visited by egg predators were observed between experimental plots although the number of predated eggs differed slightly among plants tested. Despite important differences of *D. radicum* infestation, the broccoli, the white mustard and the 'Yudal' oilseed rape genotype presented the same number of predated eggs. Our initial hypothesis that plant habitat may influence the presence of natural enemies and consequently affect the number of predated eggs could be level off by the host searching behaviour of predatory ground beetles. Indeed, in agricultural landscapes predatory ground beetles generally overcome the high variability of food resources by high dispersal rates (Woodcock *et al.*, 2010). Thus, the repartition of natural enemies should tend to be homogeneous into our field experiment. Moreover, the low number of eggs predated on the artificial egg patches could be due to the low quality of eggs in comparison to natural cabbage root fly eggs. In a 'push-pull' context, our results suggest the possibility to select plants modifying cabbage root fly infestation levels (i.e. white mustard and 'Yudal' oilseed rape) without altering their control by egg predators.

Cabbage root maggots cannot disperse over large distances therefore oviposition decisions of adult flies are essential for the development of their progeny. As many insects, if not all, the host-plant selection by *D. radicum* rely on a combination of signals integrating chemocontact, visual and olfactory cues (Schoonhoven *et al.*, 2005). Chemical compounds present on the leaf surface represent undoubtedly the final signal used by *D. radicum* for host plant acceptance (Städler *et al.*, 2002) although plant odours could be involved in synergistic effects (de Jong & Städler, 1999). Nevertheless, medium/large range information detected whilst insects are in flight play a key role to locate suitable plants in complex environments. Among the phytochemicals involved in antixenotic resistance of plants, VOCs play a critical role: plants emitting attractive blend of volatiles are merely less resistant to pests than plant releasing deterrent/arrestant signals (Smith *et al.*, 2005; Padmaja *et al.*, 2010; Hegde *et al.*, 2012). Different studies have demonstrated the role of visual (Roessingh & Städler, 1990) and olfactory cues (Finch & Skinner, 1982) in the cabbage root fly behaviour although their relative importance is still debated (Finch & Collier, 2000). However, it seems unlikely that

herbivorous insects exclusively use only one of these three signals in an ecological context (Schoonhoven *et al.*, 2005).

Our olfactometer experiments indicate that VOCs released by plants can effectively influence *D. radicum* behaviour. In comparison with pure air, the proportion of flies remaining in the first section of the olfactometer was drastically reduced when *D. radicum* were exposed to *B. oleracea*, *B. napus* ‘Yudal’ or ‘Darmor-bzh’ genotypes indicating that the three plants tested emitted attractive volatiles. Nevertheless, different levels of attractiveness can be distinguished according to the pattern of movements in the other sections. When flies were exposed to VOCs released by the ‘Darmor-bzh’ genotype, the mean time spent in the first section of the olfactometer was not significantly different than the time spent in the last section suggesting that this plant presents the lowest attractivity. Contrary to broccoli, the time spent in section 3-4 was higher than the time spent in section 1-2 when flies were exposed to volatiles emitted by the ‘Yudal’ genotype indicating that this plant is the most attractive. The rank of plant attractiveness found in the olfactometer (*B. napus* –‘Yudal’ genotype > *B. oleracea* > *B. napus* –‘Darmor-bzh’ genotype) is similar to the infestation rank observed for these three plants in the field experiment suggesting an important role of VOCs in plant colonization. Although host-plant selection may rely on a combination of stimuli, VOCs determine the ability of herbivorous insects to find a plant and consequently affect its colonization. Indeed, previous experiments carried out in field conditions have shown the possibility of modifying plant infestation through releases of synthetic VOCs (Kergunteuil *et al.*, 2012).

The present study demonstrates the possibility to select plants that could be used to redistribute cabbage root flies in broccoli crops without compromising herbivore control by natural enemies. In a ‘push-pull’ context, the white mustard *S. alba* represents a good candidate for the ‘pull’ component while the ‘Yudal’ genotype of oilseed rape is interesting to develop the ‘pull’ component. According to the literature, *B. rapa* was a promising species to attract *D. radicum* into trap areas but our results highlight the necessity to consider impact of other herbivores on plant acceptability. Obviously, additional field experiments would be required to (i) test how trap crops or intercultural plants have to be deployed and (ii) investigate whether such plant combination can effectively reduce cabbage root fly infestation in broccoli fields. Our results indicate that volatiles released by plants could influence their infestation levels by the cabbage root fly. The identification of the VOCs blends emitted by the plants tested in the present study could allow the development of a semiochemically

assisted ‘push-pull’ where trap plants would be enhanced by synthetic release of attractive VOCs in order to reduce surfaces devoted to pest control versus harvest.

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**ARTICLE N°2 : Sélection au laboratoire  
de plantes et de composés volatils  
modifiant le comportement de *Delia  
radicum*.**

*Article en préparation*





# Characterizing attractiveness of five brassicaceous plants toward the cabbage root fly, *Delia radicum*, to identify both plants and volatiles with potential for a ‘push-pull’ strategy

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## ABSTRACT

Volatiles organic compounds (VOCs) released by plants are involved in various orientation processes of herbivorous insects and consequently play a crucial role in their reproductive success. In the context of developing new strategy for crop protection, several studies have previously demonstrated the possibility to limit insect density on crops using VOCs originating either from host or non host-plants. The cabbage root fly, *Delia radicum*, is an important pest of brassicaceous crops for which control methods have to be implemented. Several studies have shown that plant odors influence the cabbage root fly behavior but only few VOCs have been identified so far. The present study aims at selecting both plants and olfactory stimuli that could be used in the development of a “push-pull” strategy against the cabbage root fly. Our olfactometer results revealed that plants belonging to the same family exhibited different levels of attractiveness toward *D. radicum*. Combining behavioral observations with results from gas chromatographic analyses of volatile profiles indicated that attractive plants emitted quantitatively more VOCs while their volatile blends were qualitatively characterized by the presence of sesquiterpenes such as  $\alpha$ -farnesene or  $\beta$ -caryophyllene. This study represents a first step to identify both attractive plants of agronomic interest and additional volatiles that could be used in trap crops to protect broccoli fields against the cabbage root fly.

**Key words:** *Delia radicum*, tubular olfactometer, plant attractiveness, volatile organic compounds, terpenoids,  $\alpha$ -farnesene,  $\beta$ -caryophyllene, behaviour-modifying strategies, “push-pull”.

## INTRODUCTION

Herbivorous insects evolve in complex environments where orientation processes are essential for their reproductive success. Plant secondary metabolism is an important source of information for several steps in the insect life cycle, such as the selection of feeding sites, shelter, mates and host plants (Visser, 1986; Schoonhoven *et al.*, 2005). Although short range visual cues and chemocontact stimuli originating from plants have been shown to play a role, long range cues conveyed by plant odours present the advantage that they can optimize behavioural responses over large distances (Schoonhoven *et al.*, 2005). The complex blends of volatile organic compounds (VOCs) released by plants may contain over 200 compounds, but only a subset of these volatiles is detected by phytophagous insects (Bruce *et al.*, 2005; Bruce & Pickett, 2011). Plant derived VOCs serve various ecological functions in plant-herbivore interactions. Herbivores can be both attracted or repelled by plant odours according to the suitability of plants and insect experiences (Visser, 1986; Wang *et al.*, 2008). In addition to host plant localization, VOCs are also used by insects to estimate resource quality (Bengtsson *et al.*, 2001; Magalhães *et al.*, 2012; Piesik *et al.*, 2013). Even in a multitrophic context, plant odours can inform herbivores about the presence of competitors or natural enemies (Sabelis *et al.*, 2001; Dicke & Baldwin, 2010). This diversity in ecological functions of VOCs emphasizes the potential to manipulate communication between plants and phytophagous insects in integrated pest management strategies. In an agricultural context these semiochemical compounds can be practically applied to reduce the use of common pesticides in integrated pest management strategies (Shrivastava *et al.*, 2010; Szendrei & Rodriguez-Saona, 2010).

During the last decade, several studies have demonstrated the possibility to limit insect density on crops with VOCs originating either from host or non host-plants (Birkett *et al.*, 2000 ; Vallat & Dorn, 2005 ; Togni *et al.*, 2010). The ‘push-pull’ strategy combines deterrent and attractant stimuli simultaneously to both deter pests from target crops and attract herbivores into trap crops surrounding the culture (Cook *et al.*, 2007). Today, this strategy has been adopted by over 30,000 smallholder farmers to protect sub-saharian cereal crops against stem borers (Hassanali *et al.*, 2008; Khan *et al.*, 2011). Successful control of stem borer populations is achieved by its response to VOCs released by plants involved in the strategy, *i.e.* the repelling inter-culture and the trap crop (Khan *et al.*, 2010). Understanding insect response to complex VOCs mixtures emitted by host and non host plants is essential for the development of new environmentally sound crop protection strategies (Pickett *et al.*, 2012).

The main purpose of the present study is to contribute to the development of a ‘push-pull’ method against the cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae). The cabbage root fly is a worldwide oligophagous pest specialized on brassicaceous plants. Females lay eggs on plant stems and larva develop by feeding on roots. This pest can cause severe damage in different brassicaceous crops such as broccoli, turnip, rutabaga, cabbage or cauliflower. Cabbage root fly infestations may cause a yield loss up to 90% in untreated fields (Finch, 1989), so, the recent limitation of insecticides toward *D. radicum* urgently requires the development of alternative crop protections. The behavioural sequence that leads to oviposition on a plant involves several stimuli. The role of proximate stimuli (*e.g.* visual cues and chemocontact stimuli) has been very well documented (Tuttle *et al.*, 1988; Roessingh & Städler, 1990; de Jong *et al.*, 2000; Finch & Collier, 2000; Griffiths *et al.*, 2001) while only a few olfactory stimuli have been identified so far, even if the distance at which they are used by *D. radicum* remains unclear and could range from 5m to 24m (Finch & Skinner, 1982). Among host plant volatiles, one compound, allyl-isothiocyanate was shown to be involved in the long distance orientation of flies (Hawkes & Coaker, 1979; Wallbank & Wheathley, 1979) while another compound, dimethyl-disulfide was shown to reduce oviposition rates (Ferry *et al.*, 2009). Also, salicylaldehyde, a common volatile emitted by various plant species, has been shown to repel flies and reduce oviposition (den Ouden *et al.*, 1997). The identification of new volatile stimuli could help developing control strategies based on pest behavior manipulations.

In the present paper, we aim at selecting both plants and olfactory stimuli that could be used in the development of a “push-pull” strategy against the cabbage root fly whether with trap crops or synthetic VOCs. First, we tested the attractiveness of five brassicaceous plants belonging to the host plant range of the fly. Based on previous studies carried out under field conditions (Kergunteuil *et al.*, in prep), three plants of agricultural interest (*Brassica oleraceae*, *B. rapa pekinensis*, *Sinapis alba*) and two genotypes of experimental oilseed rape (*B. napus* ‘Yudal’ and ‘Darmor-bzh’). The two genotypes of *B. napus* were chosen because of their contrasting resistances to different pathogens, ‘Yudal’ being generally less resistant (Manzanares-Dauleux *et al.*, 2000; Delourme *et al.*, 2008; Jestin *et al.*, 2011). We subsequently characterized their VOCs blends in order to identify compounds that could be involved in the long distance orientation of the fly and be used to attract flies in trap crop areas.

# MATERIALS AND METHODS

**Plants** The experiments were conducted on four different plant species: broccoli (*Brassica oleracea*), oilseed rape (two genotypes: *B. napus* ‘Yudal’ and ‘Darmor-bzh’), Chinese cabbage (*B. rapa pekinensis*) and white mustard (*Sinapis alba*). Single seeds were directly sown in a 9x9x11 cm pot filled with a peat: sand mixture soil (Lentse Potgrond n°2, Horticoop, Bleiswijk, The Netherlands) containing nutrients with 2 cm of clean river sand on top. Plants were grown in a glasshouse (Nijmegen, Netherlands) at 16h:8h (L:D) and 21°C:19.5°C (L:D). Daylight was supplemented with sodium lamps (600 W) when natural light was lower than 250 micromoles.s<sup>-1</sup>.m<sup>-2</sup>. Plants were watered twice a week. Plants used for experiments were 5 weeks old, corresponding to 7-8 leaves for broccoli and oilseed rape, and 10-12 leaves for Chinese cabbage. The white mustard plants grew faster and were used when they were 3 weeks old and had 7-8 leaves. All plants used for both VOC collection and behavioural experiments grew in the same conditions.

## Behavioural experiments

**Insects** Females of *Delia radicum* used for the experiments originated from a colony started in July 2012 from field collected flies (Le Rheu, Brittany, France, 48° 07’ 16’’ N, 01° 47’ 41’’ W). The fly colony was fed with a milk powder: yeast: sugar (1:1:1) mixture and kept in a climatic chamber at 16h:8h (L:D), 21±2°C and 60±10% RH. Rearing conditions were adapted from Neveu Bernard-Griffiths, 1998. Pupae from this colony were sent weekly to the Netherlands, where all the experiments took place, and kept in a cage with water and food under similar conditions. Three days after emergence, females were considered as fertilized and were used for experiments until they were 12 days old. All tests were conducted in a room maintained at 21±2°C and 60±10% RH with artificial lights. To acclimatize the flies before the experiments, they were isolated and placed in the test room at least 15 min before the assays started.

**Olfactometer tests** Behavioural responses of *D. radicum* to control (*i.e.* pure air) or odours emitted by the five test plants were monitored using a tubular olfactometer (60cm x 5cm ID) artificially divided into six notional sections of 10 cm. Light was supplied by four neon lights (Phillips, Master TL-D Reflex 36W/ 840) placed above the tube. The airflow was provided by a bottle of normal compressed air (N:0 / 80:20) moistened by bubbling the air through a

washing bottle filled with water before reaching a plant enclosed in a PET oven bag (polyethylene terephthalate, , 25x40cm, Dumil, ITH Complast BV, the Netherlands). Airflow in the olfactometer was maintained at 400ml.min<sup>-1</sup>. All connections were made with PTFE (polytetrafluorethylene) tubing. Females were placed individually in the tube and their behaviour was recorded during 10 minutes. The tubular olfactometer was placed in a white box in order to avoid any visual stimuli from the plant. For each plant genotypes, 3 or 4 individual plants per genotype and 29 to 38 females were used (between 5 and 16 per plant). Olfactometer tests were carried out over a period of three consecutive weeks. The testing of the different plant genotypes (including the control) were randomized over this period. The entire device was washed with ethanol, dried and purged during fifteen minutes with clean air before starting new observations.

## VOC analyses

**Volatile collection** Volatile compounds were collected in a climate chamber (Snijder-scientific) maintained at 21°C and 50% RH using a push-pull system as described by Tholl *et al.* (2006). The shoots were enclosed in PET oven bags connected with PTFE tubing to two pumps. A continuous air stream into the bag was generated with the first pump at 9L.h<sup>-1</sup>. The air passed an activated charcoal filter before reaching the enclosed plants while a second pump maintained the outgoing airflow at 8L.h<sup>-1</sup>. Volatiles were trapped during 24h on 30 mg of filters packed with 30 mg Porapak Q (60:80 mesh). Collection tubes were cleaned with 500 µl of gc-grade dichloromethane and dried before use. One plant of each genotype tested was sampled on the same day. Volatiles were collected from twelve individual plants for each genotype, except for the 'Yudal' genotype where eleven plants were sampled.

**Gas chromatography and VOC identification** Collected volatiles were desorbed eluting the filters with 150 µl dichloromethane containing nonyl acetate as an internal standard (10ng.µl<sup>-1</sup>). The solutions were stored at -20°C until GC-MS analysis. Qualitative and relative quantitative determination of VOCs was conducted using an Agilent 7890 Series gas chromatograph (injector temp.: 250° C, injection volume: 1µl, splitless mode) coupled to a JEOL accurate mass TOF instrument (JMS T100-GCV, interface temp.: 250 °C; ion chamber temp.: 200 °C, ionizing voltage: 70 eV) recording spectra every 0.4 s in the range from 30-500 amu. VOCs were separated with a DB-5MS column (Agilent, Santa Clara, CA, USA, 30 m, 0.25 mm, 0.25 µm) using He as carrier gas. The oven temperature was programmed as

follows: 40°C raised to 95°C (3°C.min<sup>-1</sup>), then raised to 165°C (2°C.min<sup>-1</sup>), finally raised to 250°C (15°C.min<sup>-1</sup>) and held at this temperature for 15min. After deconvolution with AMDIS (version 2.7.1), compounds were identified by comparison of retention times and mass spectra to those of authentic standards ( $\alpha$ -pinene, hexyl acetate, limonene, linalool, (Z)-3-hexenol, (Z)-3-hexenyl acetate) obtained from Sigma (St. Louis, MO, USA), or by reference spectra in the National Institute of Standards and Technology libraries (2011) and corresponding retention indices in the literature (RIs obtained on DB-5 column).

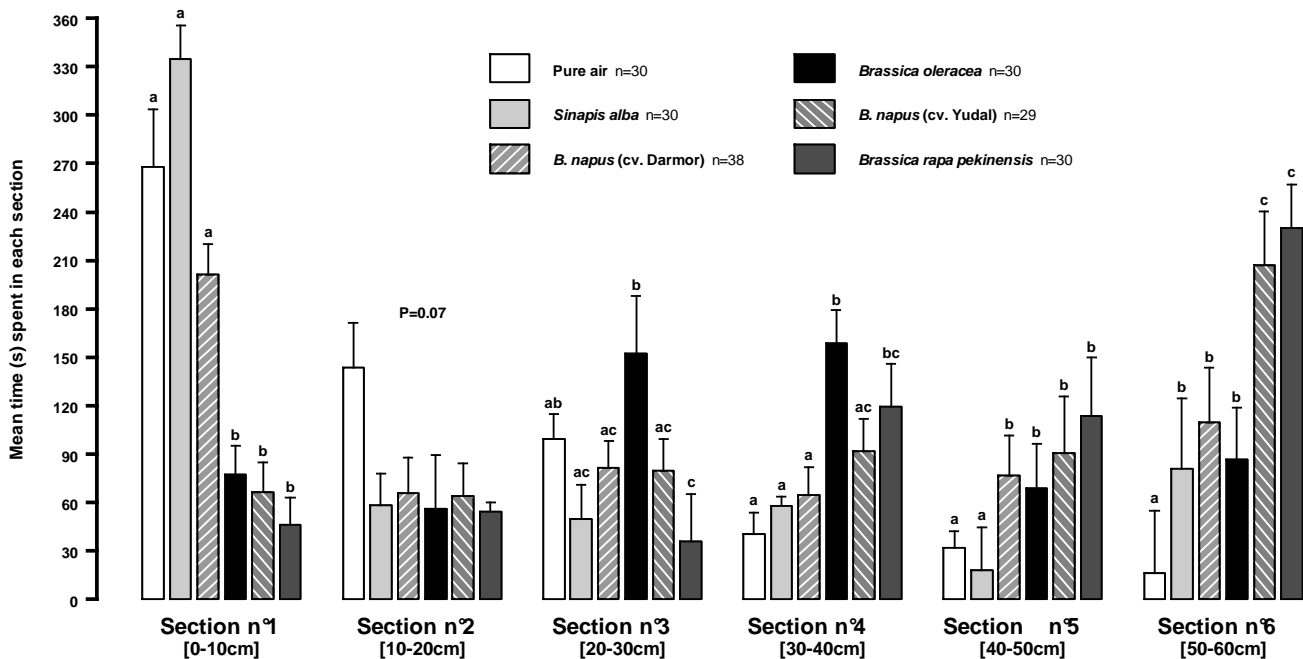
**Data analysis of chromatographic peaks** The centroided data from GC-MS were processed using the Bioconductor package XCMS (Smith *et al.*, 2006) implemented in the R software (R Development Core Team, 2011). Peaks were detected with the ‘CentWave’ algorithm (30 ppm, peak width from 2 to 20 s, s/n threshold of 3:1). Retention time correction was performed with a ‘symmetric’ method (bandwidth set to 20 seconds). As different metabolites can co-elute, the Bioconductor package CAMERA was used to group ions species according to their original compound (perfwmm parameter set to 0.6). Finally, a data matrix containing mass to charge features (m/z), retention times, mass signal intensities and peak correlation groups (*i.e.* pseudo-spectra) was generated and exported to Microsoft Excel®. VOC identification carried out previously (see above) was reported in this data table according to mass spectra and retention time. For each pseudo-spectrum, the mass signal with the highest intensity was then used as quantifier ion. In order to avoid variation in detector sensitivity, peaks were normalized to the peak area of the internal standard (quantifier ion: 126 m/z).

**Statistical analysis** All statistical tests were performed using the software R, version 2.12.2 (R Development Core Team, 2011). Mean time (s) spent in each section of the olfactometer was assessed with a generalized linear model (GLM) (package ‘lme4’). The goodness-of-fit of each model tested was estimated by graphical observations of (i) the model residuals plotted against the fitted values (ii) the quantile randomized residuals plotted against the normal distribution quantiles. A GLM with ‘quasipoisson’ distribution (linking function: ‘log’) was finally retained to take account of over-dispersion. Mean time differences were estimated by contrasts methods applied to GLM (package ‘doBy’). Differences in the relative amounts of VOCs between treatments were analysed using non-parametric tests: Kruskal-Wallis one-way analysis and pairwise Wilcoxon rank sum test (P.values were adjusted with a false discovery rate correction method). A principal component analysis (packages ‘ade4’) was performed from the whole set of relative amounts of VOCs identified and presented in the Table 1.

# RESULTS

## Behavioral experiments

In a tubular olfactometer such as the one used here, time spent by females in the first and the last section provide interesting behavioural information: while time spent in section 1 corresponds to latency or absence of response, time spent in section 6 reflects attractiveness. In pure air, *i.e.* without any plant VOCs, female flies mostly remained in the first section of the olfactometer during the test period (about 5 minutes; Fig.19). Two plant genotypes showed a similar response pattern as clean air: white mustard *S. alba* and oilseed rape *B. napus* ‘Darmor-bzh’. The time spent in the first ten centimeters of the olfactometer was not significantly different among these three treatments. However, these two plants triggered a slight upwind response as flies spent significantly more time in section 5 and/or 6 than in pure air (Fig .19).



**Figure 19** : Mean time in second ( $\pm$  SE) spent by *D. radicum* females exposed to various undamaged brassicaceous plants and to pure air in a tubular olfactometer artificially divided in 6 notional sections (section n°1: fly entrance; section n°6: entrance of airflow in the tube)

Mean times were calculated after ten minute (600 s) of observation. Significant differences in mean times are represented with different letters: GLM ‘quasipoisson’, contrasts method,  $P < 0.05$



The time spent in the first section of the olfactometer was significantly shorter than in pure air for three other genotypes: *B. oleracea*, *B. napus* ‘Yudal’ and *B. rapa pekinensis*. Volatiles from these genotypes triggered an upwind movement and flies quickly progressed towards the upwind end of the olfactometer (Fig.19). Two patterns of movement could be identified for these three attractive plant genotypes. Females exposed to broccoli volatiles spent more time in sections 3-4 than in the proximate sections 1-2 or in the ultimate sections 5-6 (GLM, contrasts method, respectively:  $t=2.98$ ,  $df=174$ ,  $P<0.01$  /  $t=2.58$ ,  $df=174$ ,  $P=0.01$ ). Females exposed to Chinese cabbage and ‘Yudal’ oilseed rape genotype volatiles spent more time in section 6 than in the others (GLM, contrasts method, respectively:  $t=-6.00$ ,  $df=174$ ,  $P<0.001$  /  $t=-4.84$ ,  $df=168$ ,  $P<0.001$ ).

**VOC analyses** Table 4 reports the 15 compounds, mainly terpenoids, found across the five plant genotypes. In addition to linalool (9), the three VOCs common to all the samples,  $\alpha$ -pinene (3),  $\beta$ -myrcene (5) and limonene (7) were authentically identified with standards. *Brassica napus* ‘Darmor-bzh’ genotype and *S. alba*, emitted less terpenoids than the others (Fig.21). These differences between plants were due to differences in quantities of both monoterpenoids and sesquiterpenes (Kruskal-Wallis one-way analysis, respectively:  $\chi^2=34.34$ ,  $df=4$ ,  $P<0.001$  /  $\chi^2=49.45$ ,  $df=4$ ,  $P<0.001$ ). Only one compound, limonene (7), was emitted in the same proportion by the five plant genotypes

**Tableau 4 :** Relative amounts of VOCs (mean  $\pm$  SE) released by undamaged shoots of five brassicaceous plants during 24h

Compounds <sup>a</sup>	Class	RI <sup>b</sup>	LRI <sup>c</sup>	<i>Sinapis alba</i> (n=12)	<i>B. napus</i> 'Darmor-bzh' (n=12)	<i>Brassica oleracea</i> (n=12)	<i>B. napus</i> 'Yudal' (n=11)	<i>Brassica rapa pekinensis</i> (n=12)
1 1-hexanol	alcohol	865.6	865	-	-	-	0.16 $\pm$ 0.04	-
2 $\alpha$ -thujene	monoterpene	927.9	929	-	0.14 $\pm$ 0.03 a	0.07 $\pm$ 0.01 b	-	-
3 <b><math>\alpha</math>-pinene</b>	monoterpene	935.0	936	0.20 $\pm$ 0.05 a	0.43 $\pm$ 0.07 b	1.11 $\pm$ 0.13 c	0.33 $\pm$ 0.06 ab	0.22 $\pm$ 0.06 a
4 $\alpha$ -phellandrene	monoterpene	974.1	971	-	0.46 $\pm$ 0.09 a	1.85 $\pm$ 0.32 b	-	-
5 $\beta$ -myrcene	monoterpene	993.3	992	0.13 $\pm$ 0.02 a	0.66 $\pm$ 0.06 b	0.25 $\pm$ 0.04 c	0.22 $\pm$ 0.05 ac	1.91 $\pm$ 0.18 d
6 <b>hexyl acetate</b>	ester	1017.7	1017	-	-	-	0.27 $\pm$ 0.14	-
7 <b>limonene</b>	monoterpene	1030.0	1029	1.65 $\pm$ 0.66 ns	1.88 $\pm$ 0.59 ns	3.18 $\pm$ 0.60 ns	1.64 $\pm$ 0.51 ns	1.79 $\pm$ 0.74 ns
8 1,8-cineole	monoterpene	1032.5	1032	-	-	2.96 $\pm$ 1.14	-	-
9 <b>linalool</b>	monoterpenoid	1105.0	1105	-	-	0.17 $\pm$ 0.04 a	-	6.05 $\pm$ 0.46 b
10 nonaldehyde	aldehyde	1106.5	1107	-	0.14 $\pm$ 0.03 ns	-	0.15 $\pm$ 0.04 ns	-
11 $\alpha$ -copaene	sesquiterpene	1367.2	1367	-	-	-	1.03 $\pm$ 0.11	-
12 $\beta$ -elemene	sesquiterpene	1383.5	1384	-	0.21 $\pm$ 0.04	-	-	-
13 $\beta$ -caryophyllene	sesquiterpene	1407.7	1409	0.37 $\pm$ 0.17 a	-	-	26.18 $\pm$ 0.12 b	2.09 $\pm$ 0.41 c
14 humulene	sesquiterpene	1434.8	1432	-	-	-	7.15 $\pm$ 0.12 a	0.53 $\pm$ 0.12 b
15 $\alpha$ -farnesene	sesquiterpene	1482.7	1484	-	-	7.41 $\pm$ 1.52 a	-	0.24 $\pm$ 0.04 b

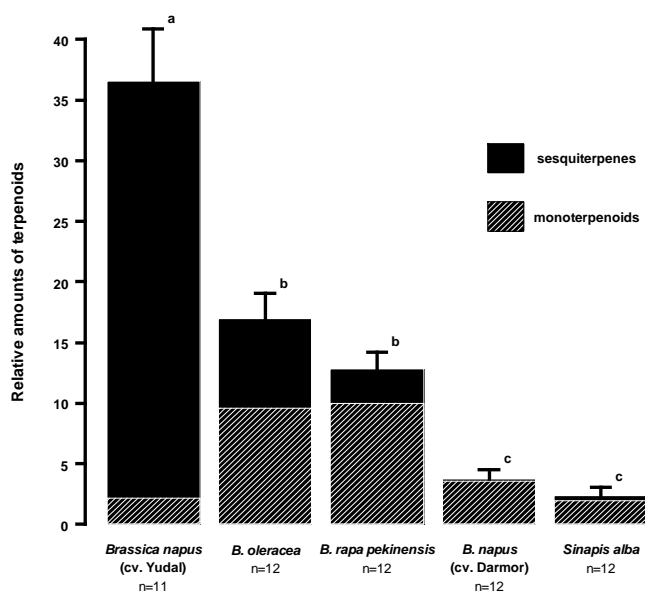
For each compound, significant differences in VOCs emissions are represented with different letters: pairwise Wilcoxon rank sum tests, p-value correction (false discovery rate),  $P<0.05$

**a.** Compounds are listed in order of their elution from a DB-5ms column and identified by mass spectra, comparison of RI from those of the literature and standard injection for compounds in bold.

**b.** RI, retention indices as determined on DB-5ms column

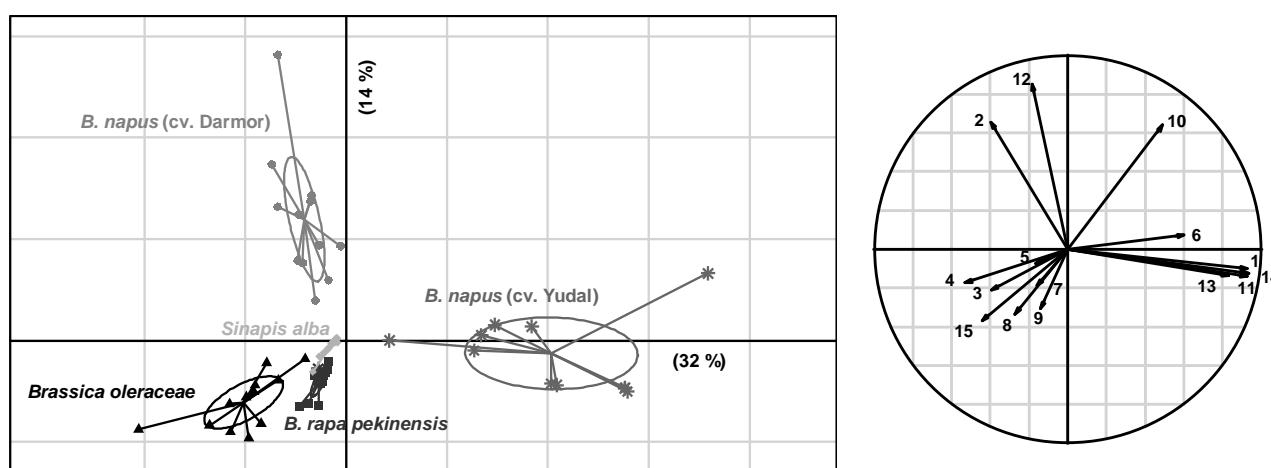
**c.** LRI, literature retention indices on DB-5ms column (<http://webbook.nist.gov>)

The overall difference between VOCs profiles was explored through a principal component analysis (Fig.21). Chemical differences in volatile blends were important both between the four species and between the two genotypes of *B. napus*. The two PCA axis explained 32% and 14% of the variance. The differences between the two genotypes of *B. napus* were pronounced on both axis of the analysis. The volatile profile of *B. oleraceae* was slightly separated from the other plants while *B. rapa pekinensis* and *S. alba* were relatively close.



**Figure 21** : Relative amounts of terpenoids (mean  $\pm$  SE) released by undamaged shoots of five brassicaceous plants during 24h

Significant differences in terpenoid emissions are represented with different letters: pairwise Wilcoxon rank sum tests, p.value correction (false discovery rate),  $P < 0.05$ . Monoterpenoids include  $\alpha$ -thujene (2),  $\alpha$ -pinene (3),  $\alpha$ -phellandrene (4),  $\beta$ -myrcene (5), limonene (7), 1,8-cineole (8), linalool (9). Sesquiterpenes include  $\alpha$ -copaene (11),  $\beta$ -elemene (12),  $\beta$ -caryophyllene (13), humulene (14) and  $\alpha$ -farnesene (15)



**Figure 20** : Multivariate analysis of volatiles collected from undamaged shoots of five brassicaceous plants

(a). Principal component analysis (PCA) of VOCs released during 24h. The percentage of explained variance for each axis of the PCA appears in parentheses

(b). Correlation circle of the PCA. The correlation circle represents the direction and intensity of correlation of original variables with the two axis of the PCA. The numbers presented on the graphic correspond to: 1.hexanol (1),  $\alpha$ -thujene (2),  $\alpha$ -pinene (3),  $\alpha$ -phellandrene (4),  $\beta$ -myrcene (5), hexyl acetate (6), limonene (7), 1,8-cineole (8), linalool (9), nonaldehyde (10),  $\alpha$ -copaene (11),  $\beta$ -elemene (12),  $\beta$ -caryophyllene (13), humulene (14) and  $\alpha$ -farnesene (15)

## DISCUSSION

Previous studies have shown that cabbage root fly orientation is partially based on plant VOCs (Hawkes & Coaker, 1979; Wallbank & Wheathley, 1979) although the role of contact cues and visual stimuli in the fly egg-laying decision is also important (Finch & Collier, 2000). The present study reveals important differences in the attraction of the cabbage root fly, a phytophagous insect specializing on brassicaceous plants, among plants belonging to the same family as well as between plant genotypes of the same species. In comparison with control, three levels of attractiveness can be distinguished for the five plants tested in our olfactometer bioassay. The attractiveness of *S. alba* and *B. napus* ‘Darmor-bzh’ genotype is limited despite the slight attraction observed in section 6 (certainly due to common plant odours). The three other plants are more stimulant; *B. oleraceae* shows an intermediate level of attraction while *B. napus* ‘Yudal’ and *B. rapa pekinensis* are the most attractive ones for the females.

Combining the behavioural observations in the olfactometer and the GC analysis indicates that flies are generally more attracted by plants emitting the highest amounts of terpenoids (*B. napus* ‘Yudal’ genotype, *B. oleraceae* and *B. rapa pekinensis*). In an ecological context, high amounts of VOCs may increase plant apparency to both natural enemies and phytophagous insects sometimes resulting in an overall increase of plant infestation (Halitschke *et al.*, 2008). Parallel experiments that we conducted in the field with the same brassicaceous plants support this hypothesis: the plants that we identified in the present study as being the most attractive also harboured the highest numbers of *D. radicum* eggs under natural conditions (Kergunteuil *et al.*, in prep). However, broccoli and Chinese cabbage trigger contrasted behavioural responses despite equal amounts of terpenoids emitted, indicating a putative role of qualitative differences for the fly response.

As shown by the principal component analysis (PCA), VOCs blends emitted by the different plants are relatively distinct. However, the three levels of attractiveness observed in the olfactometer tests are not coupled to three distinct groups of VOCs profiles. Surprisingly, the VOCs blends emitted by the two plants triggering the highest contrasts in behavioural responses, *S. alba* and *B. rapa pekinensis*, are closely projected on the PCA. The quality of a volatile signal can depend on compounds released at trace level (Birkett *et al.*, 2004) and we cannot exclude that females respond to undetected compounds. Nevertheless, the set of VOCs identified allows a good separation of the volatile blends emitted by *B. oleracea* and by the

two genotypes of *B. napus*. These plants exhibit differences for herbivore attraction suggesting that the present study allows the identification of, if not all at least some, volatile compounds involved in the cabbage root fly long distance orientation.

In our analysis, volatile blends released by undamaged plants are dominated by terpenoids. This class of compounds is certainly the largest group of secondary metabolites synthesized by plants and many olfactory receptor neurons tuned to terpenoids have been identified in herbivorous insects (de Bruyne & Baker, 2008; Degenhardt *et al.*, 2009). To our knowledge, all the compounds identified in the present study have been previously reported in the literature as being emitted by brassicaceous plants, except  $\alpha$ -copaene which is released by other plant families (Tollsten & Bergström, 1988; Ibrahim *et al.*, 2005; Abel *et al.*, 2009; Schiestl, 2010; van Dam *et al.*, 2010; Pierre *et al.*, 2011; Gols *et al.*, 2012).

The less attractive plants, *S. alba* and the ‘Darmor-bzh’ oilseed rape genotype, share only three compounds:  $\alpha$ -pinene (**3**),  $\beta$ -myrcene (**5**) and limonene (**7**). These three VOCs are also released by the other plants suggesting that no repellent compounds are released by the brassicaceous plants tested in our study. Although plants may release hundreds of different compounds, it seems that herbivores generally use only a few of them (between three and ten detectable compounds) with particular ratios in the blend to recognize host plant (Bruce & Pickett, 2011). Therefore, VOCs profiles released by the less attractive plants could be characterized by an inappropriate ratio of volatiles or by the lack of specific compounds particularly important in the attraction of the cabbage root fly.

Two monoterpenoids are specifically emitted by the plants (*B. oleracea* and *B. rapa pekinensis*) that were found attractive in the olfactometer: 1,8-cineole (**8**) and linalool (**9**). Linalool plays a role in the sub-saharian ‘push-pull’ strategy designed against stem borers. Released by host-plants and trap crops, linalool is detected by these pests and stimulates oviposition (Khan *et al.*, 2000; Birkett *et al.*, 2006). 1,8-cineole is described as toxic or repellent for various insects but it is involved in thrips attraction (Katerinopoulos *et al.*, 2005; Hosseini *et al.*, 2013). The contrasts observed for monoterpene and sesquiterpene emissions between attractive and non-attractive plants suggest that sesquiterpenes play a critical role. As indicated by the discriminant analysis only one sesquiterpene,  $\beta$ -elemene (**12**), is emitted by the less attractive plants we tested while four sesquiterpenes (**11**, **13**, **14**, **15**) are emitted by the more attractive plants. Released constitutively or induced after herbivore attacks,  $\alpha$ -copaene (**11**),  $\beta$ -caryophyllene (**13**),  $\alpha$ -humulene (**14**) and  $\alpha$ -farnesene (**15**) have been

previously described as compounds potentially involved in attraction of phytophagous insects to different plants (Magalhães *et al.*, 2012; Robert *et al.*, 2013). Among these four sesquiterpenes,  $\beta$ -caryophyllene could play an important role in attraction of the cabbage root fly. This compound is emitted in particularly high amounts by *B. napus* ‘Yudal’ genotype but also, although in lower amounts, by *B. rapa pekinensis*, the two most attractive plants in our olfactometer studies. Behavioural experiments with this compound would be necessary to confirm its attractiveness.

In the present study, two plants (*B. napus* ‘Yudal’ genotype and *B. rapa pekinensis*) have been identified as more attractive than *B. oleraceae* and could be used in the ‘pull’ component of a ‘push-pull’ strategy. So far, only Chinese cabbage is commercialized while the ‘Yudal’ genotype of oilseed rape remains unused by farmer. Nevertheless, the characterization of the VOC profiles emitted by ‘Yudal’ and ‘Darmor-bzh’ genotypes is also interesting from applied perspectives. These two oilseed rape genotypes were recently used for investigating correlations between genetical diversity and metabolomic profile (Wagner *et al.*, 2012). The identification of plant genomic regions involved in the emission of VOCs profiles altering herbivore decisions could be utilized by breeders in order to select efficient cultivars for pest behavioural-manipulation strategy. One of the main challenges in developing a ‘push-pull’ strategy against insect pests is to keep trap crop areas as small as possible for economical reasons. For this purpose, attraction of trap crop areas could be reinforced by dispensers of synthetic volatiles. Previous experiments carried out in broccoli plots are encouraging as they demonstrate the possibility of modifying plant infestation through releases of synthetic VOCs (Kergunteuil *et al.*, 2012). This study represents a first step to identify both attractive plants of agronomic interest and additional volatiles that could be used in trap crops to protect broccoli fields against the cabbage root fly.

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**ARTICLE N°3 : Utilisation de composés volatils synthétiques en plein champ pour modifier le comportement de *Delia radicum* et de ses ennemis naturels.**

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## Selecting volatiles to protect brassicaceous crops against the cabbage root fly, *Delia radicum*

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### Abstract

Volatiles resulting from plant-herbivore interactions play an important role in the behavioral decisions of phytophagous, predatory, and parasitoid insects and could be used for managing pest insects. However, to date and after about 40 years of research, documented studies on applications in the field remain extremely scarce. *Delia radicum* L. (Diptera: Anthomyiidae), the cabbage root fly, is a major pest of brassicaceous crops for which classical control strategies are currently lacking. Our previous studies showed that dimethyl disulfide (DMDS), a compound emitted by roots heavily infested by *D. radicum* larvae, was attractive for the fly's main natural enemies and could lead to a reduction of 60% in number of eggs laid on treated plants in the field. As a follow-up of this work, we conducted another field study to select additional volatiles that could be used in a push-pull approach. Several synthetic herbivore-induced plant volatiles, selected on the basis of their potential action on the behavior of both the fly and its natural enemies, were placed in odor dispensers in experimental broccoli plots and their influence on oviposition by *D. radicum* and egg predation by ground-dwelling predators was assessed. Our results confirmed the role of DMDS in reducing *D. radicum* egg numbers on broccoli plants and revealed that (*Z*)-3-hexenyl acetate, a green leaf volatile released by recently damaged plants, strongly stimulated fly oviposition. Also, two of the compounds tested slightly modified predation activity of ground-dwelling predators: acetophenone decreased the proportion of predated patches, whereas methyl salicylate increased it. This study is a first step in designing a push-pull strategy to control the cabbage root fly.

### Introduction

Volatiles resulting from plant herbivore interactions play a major role in resource foraging by predators and parasitoids. They have been shown to be involved in orientation processes, oviposition decisions, feeding site acceptance, or patch quality estimation by natural enemies (e.g., Geervliet et al., 1998; Meiners & Hilker, 2000; Dicke et al., 2003; Turlings & Wäckers, 2004; Rasmann et al., 2005; Tentelier & Fauvergue, 2007; Fatouros et al., 2008; Allmann & Baldwin, 2010). These volatiles can be used by predators or parasitoids as reliable cues to find their host

or prey and are usually considered as part of indirect defense strategies of plants (Dicke et al., 2003; Turlings & Wäckers, 2004). However, herbivore-induced plant volatiles (HIPVs) can also be used by phytophagous insects to optimize their resource use. They can be important signals to locate suitable host plants (Bolter et al., 1997; Reddy & Guerrero, 2000; Turlings & Wäckers, 2004; Halitschke et al., 2008), can stimulate oviposition (Baur et al., 1998; Agrawal & Sherriffs, 2001; John et al., 2006), host acceptance (Landolt, 1993), or feeding by larvae (Halitschke et al., 2004; Carroll et al., 2006), and serve as aggregation cues for conspecifics (Loughrin et al., 1995; John et al., 2006). On the other hand, as they are emitted once the plant has been attacked, these volatiles can also give indications of poor-quality oviposition or feeding sites (because

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of high competition or natural enemy risk) and repel herbivores (Bernasconi et al., 1998; De Moraes et al., 2001; Kessler & Baldwin, 2001; Sanchez-Hernandez et al., 2006).

The discovery of these multiple and important effects of HIPVs on insect behavior (and particularly natural enemies) opened new perspectives for managing pest insects (Degenhardt et al., 2003; Pickett et al., 2006; Turlings & Ton, 2006; Khan et al., 2008; Rodriguez-Saona & Stelinski, 2009). However, to date and after about 40 years of research on this topic, documented studies on applications in the field remain extremely scarce. The effect of synthetic HIPVs on attraction of predators and parasitoids was demonstrated in hop yards and vineyards by a series of field studies (James, 2003a,b; James & Price, 2004; James & Grasswitz, 2005). Furthermore, for one of the HIPVs [viz., methyl salicylate (MeSA)], increases in natural enemy numbers were also associated with a reduction below threshold levels of important pests like spider mites and aphids (James & Price, 2004). Field application of synthetic volatiles gave similar promising results in sweet maize, broccoli, wine grapes, and cotton (Yu et al., 2008; Simpson et al., 2011). An approach which combines the use of volatiles to manipulate both insect pests and their natural enemies has led to real applications in the field: the push–pull (sometimes also termed stimulo-deterrent diversion) strategy (Cook et al., 2007; Hassanali et al., 2008; Khan et al., 2008). This strategy is the only one practiced by farmers and is currently used for the control of the cereal stem borers *Chilo partellus* (Swinhoe) and *Busseola fusca* Fuller on cereal crops in Africa (Khan et al., 2008). It is based on the use of plant species that naturally release deterring (push) volatiles for the pests, intercropped with cereals. In addition, other plant species that release volatiles attractive to the pest (pull) are placed around the crop field. Volatiles released by plants repelling adult stem borers also appear to stimulate parasitoid foraging and result in increased levels of parasitism in the crop (Khan et al., 1997).

*Delia radicum* L. (Diptera: Anthomyiidae), the cabbage root fly, is a major pest of brassicaceous crops in countries of the north of the Holarctic region. Females aggregatively lay their eggs near plant stems and the larvae crawl in the soil to feed on the roots before pupating in the soil surrounding the roots. Root damage can result in important crop losses if pesticides are not used (e.g., Klein-Gebbinck & Woods, 2002). In northern Europe, the main insecticide against *D. radicum*, the organophosphorous chlorfenvinphos [2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate] has been banned since 2008 due to human health risks (e.g., Heudorf et al., 2004). Therefore, new strategies for controlling this severe cabbage pest are needed. A

recent study by our group investigated the potential of dimethyl disulfide (DMDS) in a control strategy against *D. radicum* (Ferry et al., 2009). This compound is emitted in large amounts by roots heavily infested by larvae and has been found to be attractive to the main natural enemies of the fly in the field (Ferry et al., 2007): carabids belonging to the genus *Metallina* (*Bembidion*), which are generalist predators of eggs and larvae of insects, and two staphylinid beetles, *Aleochara bilineata* Gyllenhal and *Aleochara bipustulata* (L.), which are both predators of the eggs and larvae of the fly and parasitoids of its pupae. Furthermore, DMDS was also found to lower the number of eggs laid on treated plants in the field by 60% (Ferry et al., 2009). These two coupled effects (i.e., repel the pest and attract its predators) opened new perspectives for utilization of DMDS in a push–pull strategy against the cabbage root fly.

As a sequel to the work of Ferry et al. (2009), we conducted another field study to select additional volatiles that could be used in a push–pull approach. Several compounds were selected on the basis of their potential action on the behavior of both the fly and its natural enemies, including (1) allyl isothiocyanate (AITC), a breakdown product of glucosinolates characteristic of *Brassica* plants (Bones & Rossiter, 2006) and found to be attractive to *D. radicum* females in wind tunnel experiments (Nottingham & Coaker, 1985), (2) MeSA, a volatile emitted after herbivore attack and attracting beneficial insects in the field in different crops, including *Brassica* (James & Price, 2004; James & Grasswitz, 2005; Orre et al., 2010; Simpson et al., 2011), (3) hexenyl acetate, a compound previously used singly or in mixtures as an attractant of herbivores and/or their natural enemies (James & Grasswitz, 2005; von Arx et al., 2011; Simpson et al., 2011), and (4) acetophenone (ACPH), a flower-derived compound attractive to fruit fly parasitoids (Rohrig et al., 2008a) and emitted by flowering plants attractive to *D. radicum* (Rännbäck, 2008). Dimethyl disulfide, which influences the behavior of the fly and its natural enemies as shown by our previous studies (Ferry et al., 2007, 2009), was also included. These volatiles were placed in experimental broccoli plots in the field and their influence on oviposition by *D. radicum* and egg predation by ground-dwelling predators was assessed.

## Materials and methods

### Field setup

Cultivated broccoli plants, *Brassica oleracea* L. var. *Italica* (cv. Marathon) (Brassicaceae), were used in the experiment. Seeds were sown individually in peat soil cylinders and grown for 6 weeks in a plastic tunnel (Thomas Plant,

Ploubazlanec, France). The experimental site was located in the 'Domaine experimental de la Motte' (INRA Center, Le Rheu, France; 48°06'07"N, 01°47'44"W). The soil was fertilized at 63.5 kg nitrogen per ha before plantation. One week later (7 April 2011), the broccoli seedlings were transplanted in the field at a density of 1.7 plants m<sup>-2</sup>. No insecticide was used, neither as a seed coating nor as a spray in the field.

We used a randomized block design consisting of four blocks. Each block included nine randomized plots corresponding to the treatments. A plot comprised 25 plants (five rows of five plants each, planted on a 0.8 × 0.8 m grid). The plots were separated from each other by four rows (i.e., 5 m) of untreated broccoli plants. Blocks were separated from each other by 5 m of uncultivated soil. Treatments consisted of two controls and seven different volatiles supplied by means of odor dispensers (Natural Plant Protection, Pau, France) positioned at the center of the plots (Figure 1).

#### Volatiles tested

Volatiles likely to play a role in the behavior of the cabbage root fly or its natural enemies were tested: AITC, (Z)-3-hexenyl acetate (HA), MeSA, ACPH, and DMDS. Two additional treatments were originally planned but later excluded from the analyses: Z-3-hexenol was out of supply during the experiment and benzaldehyde crystallized inside the dispensers and did not volatilize properly.

Products were obtained from Sigma-Aldrich (St Louis, MO, USA). Pure product (2–3 ml) was deposited in the dispensers. Depending on the product volatility dispensers were replaced every week (AITC, DMDS) or every other

week (HA, MeSA, ACPH). Two dispensers were used for all volatiles except for MeSA, which is not very volatile and was applied using four dispensers per plot. The quantity of product volatilized was checked by weighing the dispenser before it was installed in the field and after it was replaced. The mean volatility of the compounds was 95.00 ± 9.54 (AITC), 26.43 ± 2.15 (MeSa), 31.75 ± 7.70 (HA), 31.75 ± 3.36 (ACPH), and 234.00 ± 7.76 mg per day (DMDS).

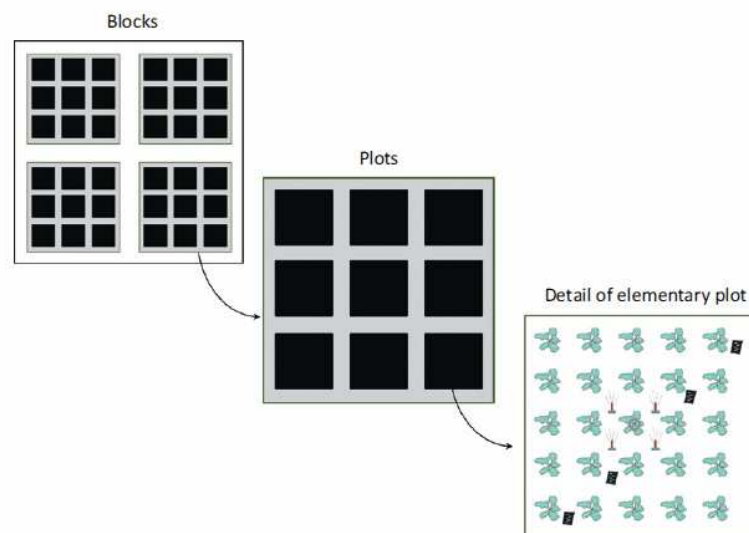
#### Oviposition

Egg laying by *D. radicum* was monitored using felt traps (for a description, see Bligaard et al., 1999) positioned around the stem of the plants, where flies deposit their eggs. These traps are used by farmers as an indicator of pest prevalence. Felt traps were placed on one plant in the middle of each plot surrounded by the odor dispensers (Figure 1). Traps were collected every week and the eggs found inside were counted and removed. Emptied traps were then replaced on the same plants (or on the nearest plant when this plant had died). Egg deposition was monitored from 12 April to 10 May 2011.

#### Egg predation

Egg predation in the plots was assessed using artificial patches of eggs. These patches consisted of a 1-cm<sup>2</sup> piece of black paper pinned down to the soil surface and protected from the rain using a small plastic cover. Five *D. radicum* eggs collected in our rearing facility were glued onto the paper with gum arabic. Four patches were placed next to the stem of four plants in each elementary plot and maintained in the plots for 48 h, after which egg predation was recorded. We measured the percentage of patches in

**Figure 1** Schematic representation of the experimental field consisting of four blocks as repetitions, each block of nine randomized plots corresponding with the nine treatments. A plot comprised 25 plants (five rows of five plants each, planted on a 0.8 × 0.8 m grid). The plots were separated from each other by four rows of untreated broccoli plants. Blocks were separated from each other by 5 m of uncultivated soil. Treatments consisted of two controls and seven different volatiles supplied by means of odor dispensers. Two to four dispensers were positioned at the centre of the plots. ○ felt trap; ■ artificial egg patch; ⊥ odor dispenser; 🌱 broccoli plant.





which predation occurred (at least one egg predated per patch) and the mean number of predated eggs when predation occurred. This experiment was repeated in two consecutive weeks (starting on 2 and 9 May). The presence of *Aleochara* spp. in the experimental field was assessed using two pitfall traps placed in each block and monitored weekly. Predation tests were carried out when more than 15 adults were found per trap.

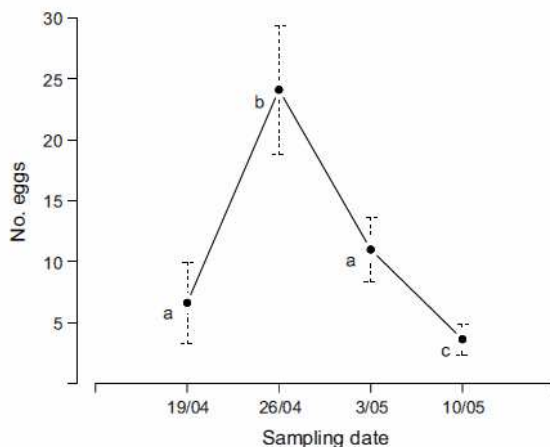
#### Statistical analysis

All statistical analyses were carried out with R software, version 2.12.1 (R Development Core Team, 2010). The number of *D. radicum* eggs laid and the number of eggs predated on in the artificial patches were analyzed using generalized linear mixed models (GLMM) with a Poisson distribution for errors and using blocks as a random factor. Comparison between treatments was carried out using an analysis of contrast (function 'esticon' of the package 'doBy'; Højsgaard, 2004). Egg predation was also analyzed by comparing the proportion of artificial patches where predation occurred with a  $\chi^2$  test (basic assumption: balanced proportion of artificial patches predated and not predated) followed by an exact binomial test (function 'binom.test',  $P = 0.5$ ).

## Results

### Oviposition

*Delia radicum* colonized experimental plots soon after plantation and the number of eggs per trap in control plots

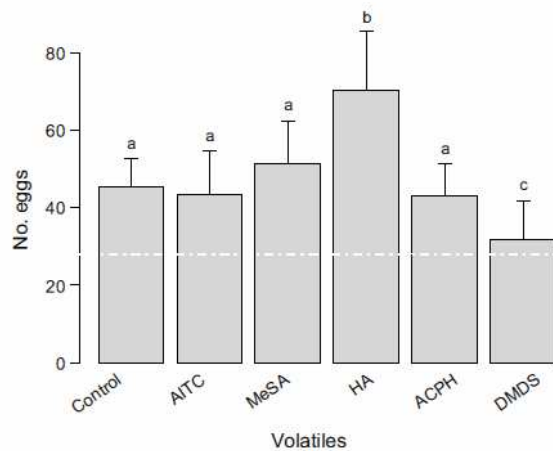


**Figure 2** Mean ( $\pm$  SE) number of *Delia radicum* eggs found per felt trap on control broccoli plants on four sampling dates. Broccoli seedlings were transplanted in plots on 7 April. Means with different letters are significantly different (GLMM Poisson and analysis of contrast:  $P < 0.05$ ).

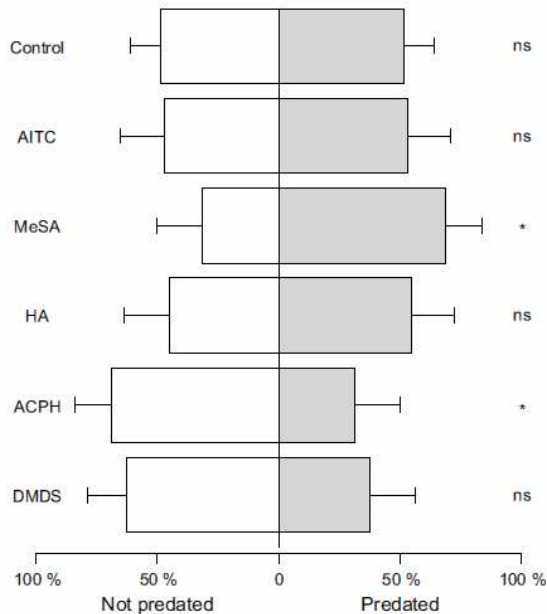
rapidly exceeded the threshold level of seven eggs per felt trap per week, which is habitually used by farmers to decide whether or not to spray against the pest (Figure 2). The presence of two synthetic HIPVs influenced the egg laying activity of *D. radicum* on broccoli plants (GLMM:  $\chi^2 = 46.71$ , d.f. = 5,  $P < 0.001$ ; Figure 3). (*Z*)-3-hexenyl acetate significantly increased the number of eggs laid on plants (GLMM:  $\chi^2 = 5.99$ , d.f. = 1,  $P < 0.05$ ), whereas DMDS significantly decreased it (GLMM:  $\chi^2 = 7.00$ , d.f. = 1,  $P < 0.01$ ). The other compounds, AITC, MeSA, and ACPH, did not modify the number of eggs laid compared to the control (GLMM:  $P > 0.1$  for each comparison with control). The number of eggs was well over the threshold of seven eggs per felt trap per week (i.e., 28 eggs per month) for all treatments except for DMDS (Figure 3).

### Egg predation

The presence of some synthetic HIPVs influenced predation activity of *D. radicum* eggs on artificial patches ( $\chi^2 = 11.47$ , d.f. = 5,  $P < 0.05$ ; Figure 4). Acetophenone significantly decreased the proportion of predated patches (10 patches predated upon out of 32 in total; exact binomial test:  $P < 0.05$ ), whereas MeSA significantly increased it (22 patches predated upon out of 32 in total; exact binomial test:  $P < 0.05$ ). The other compounds, AITC, DMDS,



**Figure 3** Mean ( $\pm$  SE) cumulative number of *Delia radicum* eggs found per felt trap on four sampling dates (19 and 26 April, 3 and 10 May) in control plots and plots where various synthetic herbivore-induced plant volatiles (HIPVs) were released: allyl isothiocyanate (AITC), methyl salicylate (MeSA), (*Z*)-3-hexenyl acetate (HA), acetophenone (ACPH), or dimethyl disulfide (DMDS). Volatiles were supplied with odor dispensers placed in the middle of the plots. The dashed line corresponds to the threshold of 28 eggs per felt trap per month, which is habitually used by farmers to decide whether or not to spray against the pest. Means with different letters are significantly different (GLMM Poisson and analysis of contrast:  $P < 0.05$ ).



**Figure 4** Mean (+ SE) percentage of *Delia radicum* artificial egg patches predated in control plots and plots to which various HIPVs were added: allyl isothiocyanate (AITC), methyl salicylate (MeSA), (*Z*)-3-hexenyl acetate (HA), acetophenone (ACPH), or dimethyl disulfide (DMDS). Volatiles were supplied with odor dispensers placed in the middle of the plots. \* $P < 0.05$ ; ns, not significant (exact binomial test).

and HA, did not influence the proportion of predated patches. However, none of the HIPVs significantly influenced the number of eggs predated on artificial patches (GLMM:  $\chi^2 = 0.76$ , d.f. = 5,  $P > 0.05$ ). The mean number of eggs predated on all plots was  $2.87 \pm 0.14$ .

## Discussion

The role of HIPVs in the behavioral decisions of phytophagous, predatory, and parasitoid insects has been demonstrated in many laboratory studies. However, demonstrations of the usability of HIPVs to manipulate pest and natural enemy behavior in the field and to reduce crop damage remain scarce (but see Khan et al., 2008). In the present paper we aimed at selecting volatiles that could influence the behavior of both a pest of brassicaceous crops, *D. radicum*, and its main natural enemies in the field. Our results confirmed the role of DMDS in reducing egg laying by *D. radicum* on broccoli plants but also revealed that HA strongly stimulated oviposition by this fly in the field. Moreover, some of the test compounds slightly modified (either positively or negatively) the predation activity of ground-dwelling predators.

Among the HIPVs tested in our experiments, HA was the only compound that stimulated oviposition by *D. radicum*. (*Z*)-3-hexenyl acetate is a green leaf volatile emitted by many plants including cabbage crops (Reddy & Guerrero, 2000; Shiojiri et al., 2001, 2010) and it is attractive to other pests of cabbage crops such as the diamondback moth, *Plutella xylostella* (L.), in the field (Reddy & Guerrero, 2000; Dai et al., 2008). To our knowledge, it is the first report of HA to have an effect on the behavior of *D. radicum*.

In *D. radicum*, several compounds present on the surface of cabbage plants are perceived by specific receptor cells in the tarsal sensilla and they have been shown to stimulate oviposition. These compounds include glucosinolates and thia-triaza-fluorenes (Roessingh et al., 1992; Hurter et al., 1999; Gouinguéné & Städler, 2006). Host plant acceptance by the cabbage root fly seems to result from a synergistic response to simultaneously perceived olfactory and contact chemostimulation (de Jong & Städler, 1999). The presence of HA could have enhanced the effect of the contact cues, naturally present on broccoli plants. Further laboratory experiments need to be performed to determine whether this compound attracts *D. radicum* or stimulates (alone or in combination with other known compounds) its oviposition.

Surprisingly, plants surrounded by odor dispensers containing AITC did not receive more *D. radicum* eggs than control plants. This volatile results from the hydrolysis of glucosinolates and is typically released by damaged brassicaceous plants containing high levels of 2-propenyl glucosinolate or sinigrin (Bones & Rossiter, 2006). It was previously shown to be attractive to the cabbage root fly and has been used in bait traps to monitor populations (adults) in the field (Wallbank & Wheatley, 1979; Finch & Skinner, 1982; Nottingham & Coaker, 1985) but has never been shown to influence oviposition. Methyl salicylate and ACPH did not have any effect on plant infestation levels either. Although all these compounds could have no effect on *D. radicum* oviposition behavior, it is also possible that we applied biologically irrelevant concentrations. To avoid differences in compound volatility using solvents, we placed pure products in our dispensers and obtained different evaporation rates for each compound, but rather stable for a given compound. Variations in concentrations of volatile compounds have been shown to influence the behavior of *D. radicum* in previous studies (Wallbank & Wheatley, 1979; Ferry, 2007). Therefore, concentrations other than those evaluated here might have different effects on the behavior of the fly.

Consistent with our previous studies, DMDS reduced egg laying by *D. radicum*, although we applied higher doses here. This compound is emitted by brassicaceous

roots heavily infested by *D. radicum* larvae for an extended period of time (i.e., 3 weeks). Therefore, it may be an indicator of a poor-quality oviposition site and play an important role in patch quality assessment by the fly. Reducing clutch sizes on plants emitting high amounts of DMDS may be adaptive for *D. radicum* as it helps reducing intraspecific competition among larvae and can also avoid choosing plants providing limited food resources for the offspring. Moreover, as it attracts the main predators of the fly (Ferry et al., 2007), DMDS may also indicate a high predation risk for the eggs. Our results confirm the importance of DMDS in reducing infestation levels of brassicae plants in the field.

As for natural enemy activity in the field in HIPV-enriched broccoli plots, we were only able to demonstrate marginal effects. Methyl salicylate tended to stimulate predation activity, whereas ACPH tended to decrease it. These two compounds modified predator visits to artificial egg patches but none of the compounds tested influenced the number of eggs predated in these patches. Although some studies demonstrated the impact of artificial HIPVs on natural enemy recruitment in the field, studies documenting the effect of these compounds on predatory activity are still scarce (Khan et al., 2008; Rodriguez-Saona et al., 2011). Yet, predation is of utmost importance for crop protection. Here we focused on the influence of artificial odors on predation activity, rather than on natural enemy presence.

Previous laboratory experiments have shown that one of the compounds tested here, DMDS, can stimulate egg predation by *A. bilineata* (Ferry, 2007). However, this effect could not be confirmed in the field, neither here nor in previous experiments (Ferry et al., 2009). It highlights that laboratory studies conducted under controlled conditions cannot always be validated under field conditions. The absence of any effect of artificial HIPV on predation levels in the field could be due to disruption of natural enemy behavior because of false information: artificial HIPVs could indicate the presence of high levels of prey resource but actual resources present may not match the signal perceived. Also, the artificial egg patch setup we used may not be optimal for testing activity of ground-dwelling predators in the field as less than 50% of *D. radicum* eggs were eaten in all our treatments. Indeed, egg patches were placed at some distance from odor dispensers and they may have been difficult to find if predators first oriented toward the artificial odor sources because the detectability of isolated eggs may be very low. Our results may also indicate that all the odors tested, except MeSA, do not enhance global prey searching in the field. Methyl salicylate is by far the most tested HIPV in field studies for its influence on natural enemy attraction (Rodriguez-Sa-

ona et al., 2011). It has been shown to attract a wide range of predatory and parasitic arthropods in as many as nine crops and is considered as a broad-spectrum attractant (Rodriguez-Saona et al., 2011). However, the influence of MeSA on ground-dwelling predators has rarely been tested.

*Delia radicum* females are synovigenic and need to feed on carbohydrates and proteins to mature their eggs (Finch & Coaker, 1969; Finch, 1971). These nutrients can be found on flowering plants surrounding brassicae crops and are regularly visited by females (Finch & Coaker, 1969). Acetophenone is emitted by flowering plants including alyssum, *Lobularia maritima* (L.) Desv. (Rohrig et al., 2008a), a brassicae species of which the flowers are particularly attractive to *D. radicum* females (Rännbäck, 2008). In our experiments, we found no effect of ACPH on egg laying by *D. radicum* but this compound negatively affected the activity of ground-dwelling predators. Such an effect has not been described before, but this compound has been found attractive to some parasitoid species (Rohrig et al., 2008a) and *L. maritima* has been shown to attract numerous species of parasitoids and predators of several insect pests under field conditions (Rohrig et al., 2008b; Sivinski et al., 2011).

This study is a first step in designing a push-pull method to control the cabbage root fly, but many steps need to be investigated before it can be implemented. We have found two volatiles with opposing effects on the oviposition behavior of the fly in the field and one that slightly enhanced predation activity. The next step would be to combine them in the field in a push-pull type disposition – i.e., DMDS and MeSA in the target crop, hexenyl acetate in neighboring field edges – to check whether they can be used to limit crop damage in broccoli fields. In parallel experiments (A Kergunteuil, S Dugravot & AM Cortesero, unpubl.), we also investigated the effect of several species and varieties of Brassicaceae on fly and natural enemy behavior. Like for the synthetic compounds tested here, we have found plants that stimulate egg laying and others that reduce egg laying by *D. radicum*. The addition of both specific plant species and synthetic volatiles affecting the colonization of plants by the pest would be interesting to combine in future field experiments.

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**ARTICLE N°4 : Analyse spatiale de l'influence des composés volatils de synthèse sur l'infestation de plantes par *Delia radicum*.**

*Article en préparation*





# Spatial effect of synthetic volatiles on field colonization by the cabbage root fly

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## ABSTRACT

Several studies have demonstrated the potential of volatile organic compounds (VOCs) released by plants for developing integrated pest management strategies based on behavioural modifications. However, these studies have paid very limited attention to spatial effects despite their importance for VOC use in the field. Determining how distance from volatile source influence behaviour of pests and natural enemies is essential to determine how VOCs have to be deployed in the field and optimize behavioural manipulations. Our previous results showed that VOCs can influence the behaviour of the cabbage root fly (*Delia radicum*) and its egg predators in the close vicinity of the dispensers. In the present study we extended observations to different distances around volatile point-sources and assessed the level of infestation in the field. For this purpose, we monitored over three distances from the dispensers (0.50m, 1m50 and 2m50) the effect of seven synthetic VOCs on (i) *D. radicum* oviposition during the plant growing season, (ii) the number of pupae found per plant after the season and (iii) the parasitization level of these pupae. Our study showed that the VOCs tested differentially affected cabbage root fly oviposition within broccoli plots depending on distance from the dispensers and confirmed the potential of dimethyl disulfide, (Z)-3-hexenyl acetate and methyl salicylate to develop a “push-pull” strategy against the cabbage root fly. Our investigations on the effect of synthetic VOCs along a small spatial transect in the field outline challenges that will need to be resolved before actual applications.

**Key words:** synthetic VOCs, behavioral modification, spatial effects, *Delia radicum*, dimethyl disulfide, (Z)-3-hexenyl acetate, methyl salicylate, “push-pull” strategy.

# INTRODUCTION

All plants release a wide array of volatile organic compounds (VOCs) in their surrounding environment. Several organs such as flower, leaves, roots, bark and specialized tissues (*e.g.* trichomes) emit plant volatiles (Holopainen & Blande, 2012). While 200 compounds may compose a volatile blend released by an individual plant, over 1700 VOCs have been identified across 90 plant families (Dudareva *et al.*, 2006, Bruce & Pickett, 2011). In an ecological context, this chemical diversity leads to a broad diversity of signals that are highly involved in the architecture of food web (Heil, 2008; Hare, 2011; Kaplan, 2012 a).

Plants are characterized by a sessile life where volatiles are crucial to communicate. In response to phytophagous attacks, plants emit herbivore-induced plant volatiles (HIPVs) (Holopainen & Blande, 2012). Among plant-plant communication, HIPVs can be detected by undamaged plants neighboring the infested one to prime their own defenses against subsequent attack of herbivores. This so-called ‘priming effect’ can occur between both conspecific and heterospecific plants and can reinforce antibiosis resistances when the attack is effective (Engelberth *et al.*, 2004; Kessler *et al.*, 2006; Ton *et al.*, 2006).

Moreover, plant volatiles can directly influence insect behavior through plant-insect communication. The VOCs released by plants can be used by herbivorous insects to adjust oviposition (de Jong *et al.*, 1999) or feeding at the larval stage (Cobb, 1999) and also play a key role in finding resources such as mates (von Arx *et al.*, 2012) or host plants (Bruce & Pickett, 2011). In addition, variations in VOC profiles can be used by herbivores to estimate resource quality according to plant phenology (Szendrei *et al.*, 2009; Magalhães *et al.*, 2012) and previous stresses (Pinto *et al.*, 2010; Piesik *et al.*, 2013). In a multitrophic context, HIPVs also inform herbivores about the presence of competitors or natural enemies and consequently modify their behaviour (Sabelis *et al.*, 2001). Also, HIPVs are involved in the recruitment of natural enemies (Dicke & Sabelis, 1988; Turlings *et al.*, 1990) and are often considered as part of indirect defence strategies of many different plant species (Mumm & Dicke, 2010).

Over the last ten years, the understanding of the ecological functions served by VOCs has resulted in a striking increase of investigations about their potential in integrated pest management strategies (Rodriguez-Saona & Stelinsky, 2009). In this context, synthetic VOCs can be used in the field with different objectives: induction of plant defence in target crop (Bernasconi Ockroy *et al.*, 2001; von Mérey *et al.*, 2011), disruption of host plant finding by

herbivores (Reddy & Guerrero, 2000; Martel *et al.*, 2007) or recruitment of natural enemies (James, 2005; Simpson *et al.*, 2011). The ‘push-pull’ strategy relies on synergistic effects between two or more of these approaches. While the ‘push’ component aims at reducing pest density in the main crop, the ‘pull’ component concentrates its populations in limited areas facilitating control by natural enemies. For this purpose, several types of semiochemicals can be used including VOCs that may act over long distances (Cook *et al.*, 2007). . However, strategies based on the release of VOCs have paid very limited attention to spatial effects while they are likely to play a crucial role. Indeed, these spatial effects determine how synthetic volatiles have to be deployed in the field in order to optimize behavioural manipulation of pests and natural enemies. Spatial effects of synthetic VOCs could be due to a simple dose-dependent functional response of insects but could also result from their redistribution in the field. Indeed, for a constant density of insects at the field scale, areas where VOC concentrations are attractive could increase insect density at the expense of adjacent areas where insect density could be reduced (Braasch & Kaplan, 2012).

The cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae), is a worldwide pest specialized in brassicaceous plants and reducing yields in many crops such as cauliflower, turnip, rutabaga and broccoli (Finch, 1989; Meyling *et al.*, 2013). Females lay eggs on the soil close to plant stems and larva cause agronomic damages by feeding on the roots. Ground dwelling egg predators including carabidae and staphylinidae play an important role in the natural control of *D. radicum* (Coaker & Williams, 1963; Prasad & Snyder, 2004). Larvae are frequently parasitized by the hymenopteran, *Trybliographa rapae* while two species of staphylinidae, *Aleochara bilineata* and *A. bipustulata*, parasitize the pupae. Due to the relative immobility of cabbage root maggot, host-plant selection by adult flies is crucial for the successful development of the larvae. Although chemocontact stimuli play an important role in host plant acceptance (Städler *et al.*, 2002), females also use long range signals such as visual (Roessingh & Städler, 1990) and olfactory cues (Traynier, 1967; Hawkes & Coaker, 1979; Wallbank & Wheatley, 1979) to optimize host plant finding. Among host plant volatiles used by *D. radicum*, allyl-isothiocyanate was early shown to be involved in the long distance orientation of flies (Finch & Skinner, 1982) while dimethyl-disulfide was shown to reduce oviposition rates (Ferry *et al.*, 2009) and (Z)-3-hexenyl acetate to strongly increase these rates (Kergunteuil *et al.*, 2012). However, the distance at which VOCs are used by the cabbage root fly remains unclear. Although, Finch & Skinner, 1982 suggested that flies respond to plant volatiles from a distance ranging from 5m to 24m, more recently Finch & Collier, 2000

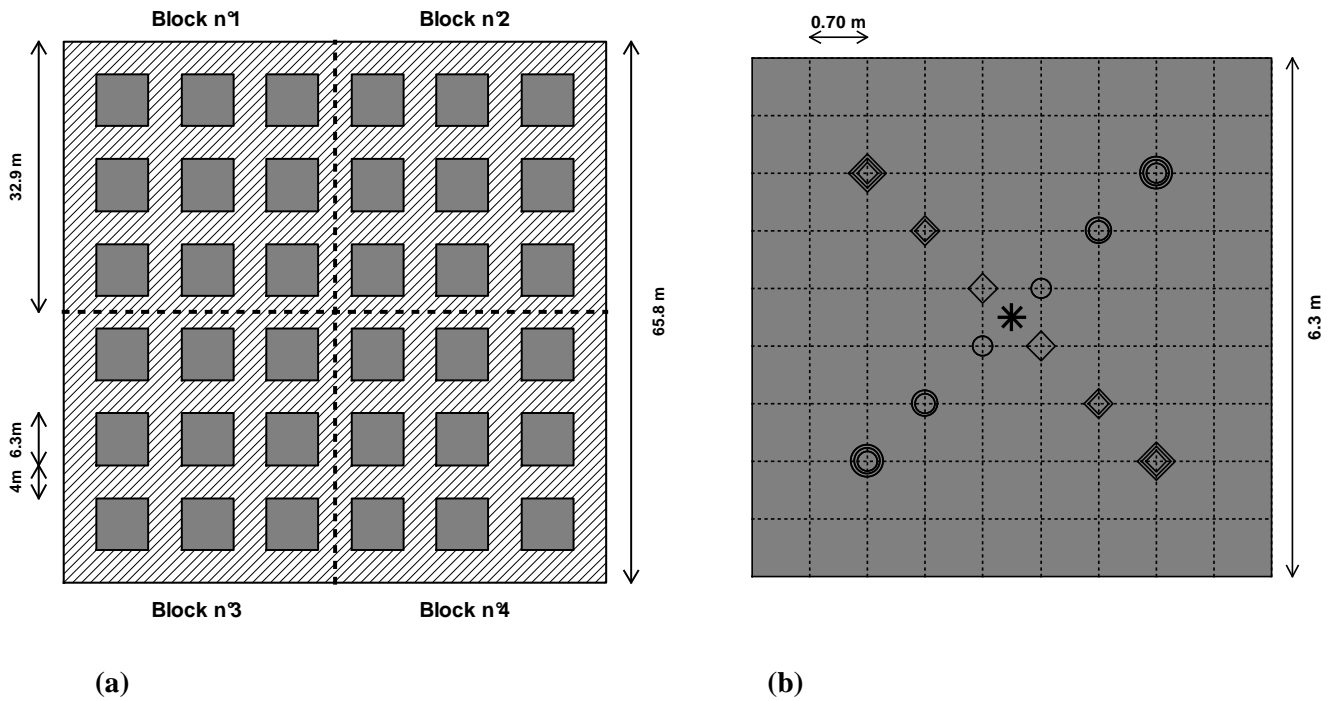
considered VOCs as cues detected when flies are passing over suitable host plants and stimulating *D. radicum* arrestment before landing.

A previous experiment conducted in broccoli fields demonstrated that both oviposition of *D. radicum* and behaviour of egg predators were altered in the close vicinity of dispensers releasing synthetic VOCs (Kergunteuil *et al.*, 2012). However, this study did not consider spatial effects of the volatiles released and did not assess resulting plant infestation levels. Such information is needed before using these VOCs to protect broccoli crops against the cabbage root fly. In the present study, we aim at understanding how synthetic volatiles should be deployed in a field in order to develop a “push-pull” strategy against this pest. For this purpose, we monitored over three distances from the dispensers the effect of seven synthetic VOCs on (i) *D. radicum* oviposition during the crop growing season, (ii) the number of pupae found per plant at the end of the season and (iii) the parasitization level of these pupae. Among the VOCs tested in previous field experiments, two volatiles were shown to affect the attack rate of egg predators, acetophenone (ACPH) and methyl salicylate (MeSA). Near the dispensers, one volatile increased the cabbage root fly oviposition, (*Z*)- 3- hexenyl acetate (HA) while another volatile reduced the number of eggs laid, dimethyl disulfide (DMDS). Despite a lack of response for allyl isothiocyanate (AITC) in the previous field experiment we decided to test it again according to the high number of studies which have suggested the attractive effect of AITC (Hawkes & Coaker, 1979; Wallbank & Wheathley, 1979; Finch & Skinner, 1982; Nottingham & Coaker, 1985). Additionally, two other VOCs, limonene and carvone were retained in the present study as they have been shown to repel diamond back moth, another important pest of brassicaceous crops (Ibrahim *et al.*, 2005), and to a lesser extent cabbage root maggots (den Ouden *et al.*, 1996).

## **MATERIALS AND METHODS**

### **Field experiment**

The field experiment was performed during spring 2012 in broccoli fields (*Brassica oleraceae* L. var. *Italica* cv. ‘Marathon’) located at the experimental station of “La Motte” (INRA Center), l’Hermitage, Brittany, France (48°08’30’’ N, 1°47’01’’ W). Broccoli plants were sown individually in peat soil cylinders and grown for 6 weeks in a plastic tunnel before



**Figure 26.** Experimental field setup

(a) Schematic representation of the experimental field consisting of 4 blocks as repetitions. Each block contained 9 randomized plots (2 controls and 7 treatments) separated from each other by 4m of bare soil. (b) A plot comprised 100 plants (10 rows of 10 plants each, planted on a 0.7m x 0.7m grid). Synthetic VOCs were released by three dispensers (asterisks) positioned at the centre of the plots. Felt traps (circles) and plants used for pupa collection (diamonds) were positioned at three distances from the dispensers: 0.50m (one symbol), 1m50 (two symbols) and 2m50 (three symbols).

plantation (commercial provider: Thomas Plants, Ploubazlanec, France). The field was cultivated according to usual agricultural practices and no pesticides were applied. We used a randomized block design consisting of 4 blocks (1 082.4 m<sup>2</sup>) of uncultivated soil (Figure 26). In each block, 9 experimental plots (10x10 plants, 39.7 m<sup>2</sup>) of broccoli were planted the 3rd of April on a 0.70x0.70m grid. Treatments consisted of 2 controls without synthetic volatiles released and 7 plots with different volatiles supplied by odor dispensers (Natural Plant Protection, Pau, France) positioned the 4th of April at the centre of the plots.

## Volatiles tested

Different volatiles released by brassicaceous plants or generally distributed among plants and likely to play a role on the cabbage root fly or its natural enemies were tested: acetophenone (ACPH), dimethyl disulfide (DMDS), allyl isothiocyanate (AITC), limonene, (Z)-3-hexenyl acetate (HA), methyl salicylate (MeSA) and carvone. Products were obtained from Sigma-Aldrich, St Louis, USA. Two to 3 ml of pure products were deposited in the dispensers and three dispensers were placed in each experimental plot. In order to assess diffusion rates (see appendix) dispensers were weighted and replaced weekly.

## Data collection

**Cabbage root fly oviposition** Egg laying by *D. radicum* was monitored using felt traps (for a description, see Bligaard *et al.*, 1999) positioned around the stem of the plants, where flies deposit their eggs. In each plot, felt traps were placed on six plants positioned at three distances to the dispenser: 0.50m, 1m50 and 2m50 (Figure 1). Traps were collected every week and the eggs found inside were counted and removed. Emptied traps were then replaced on the same plants (or on the nearest plant when this plant had died). The presence of eggs was checked from the week following the plantation until the oviposition period of the first generation of *D. radicum* ended (i.e. from May 1st to May 22nd).

**Pupae collection and emergences** Pupae were collected in the field on June 18th, *i.e.* four weeks after the end of the oviposition period of *D. radicum*, thereby allowing a complete larval development of the eggs present. In each plot, pupae were sampled on six plants distributed along a diagonal opposed to the plants used for egg monitoring (Figure 1). Thus, three distances from the dispensers were sampled: 0.50m, 1.50m and 2.50m. Approximately two liters of soil (15-20 cm deep, 20cm of diameter from the stem) from around the roots of selected plants was collected, stored in a cold chamber (4°C) and maintained in the dark until the collection of pupae. Each sample was washed in water and pupae were separated from the soil by flotation. Pupae were then held at 20±2°C, 55±5 RH, 16h: 8h (L: D) until insect emergence.

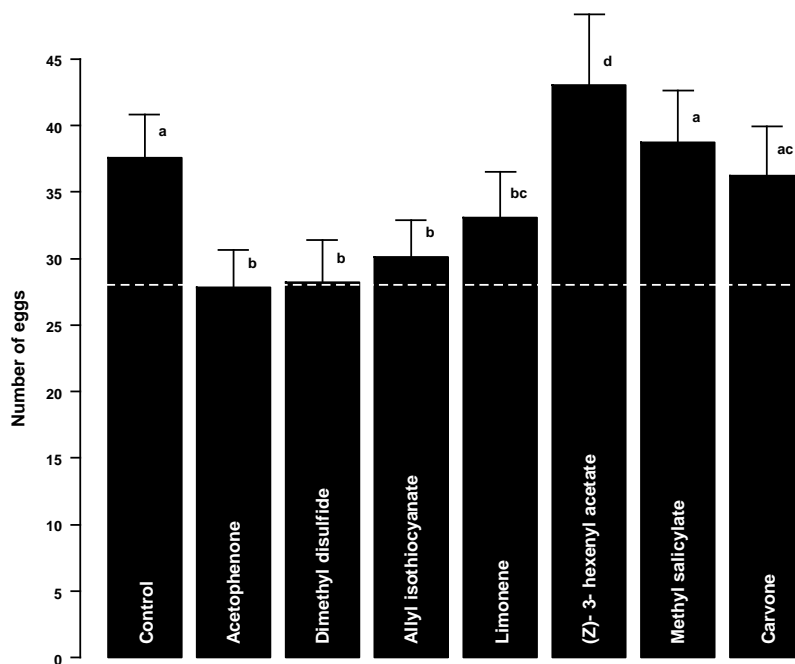
## Statistical analyses

All statistical analyses were carried out with R software (R development core team, R version 2.12.1). Analyses about cabbage root fly oviposition were conducted on the cumulative number of eggs laid per plant over the four sampling weeks. The number of *D. radicum* eggs laid and the pupae found per plant were analyzed using two generalized linear mixed models (GLMM, package ‘lme4’) for Poisson data (linking function: ‘log’) considering ‘volatile released’, ‘distance sampled’ and their interaction as fixed factors, with spatial ‘block’ included as random factor. The effect of each fixed factor was assessed by likelihood ratio tests. After verification for the influence of the fixed factor studied, comparisons between treatments were carried out using an analysis of contrast (package ‘doBy’) and p.values were adjusted by a false discovery rate correction method. The proportions of adults

emerging from collected pupae were compared by a pairwise chi-square test ( $\chi^2$ ) and p-values were corrected using a false discovery rate method.

## RESULTS

**Cabbage root fly oviposition** At the plot scale, synthetic volatiles released significantly influenced *D. radicum* infestation (GLMM, Poisson data, likelihood ratio test:  $\chi^2=163.57$ , 7 d.f.,  $P<0.001$ ). In comparison to control, four volatiles, ACPH, DMDS, AITC



**Figure 27 :** Mean ( $\pm$  SE) cumulative number of *Delia radicum* eggs per plant. Number of eggs found per felt trap was cumulated over four consecutive sampling dates (1, 8, 15, 22 May). The dashed line corresponds to the threshold of 28 eggs per felt trap per month, which is habitually used by farmers to decide whether or not to spray against the pest. Means with different letters are significantly different: GLMM 'poisson', analysis of contrast and p.value correction (false discovery rate),  $P<0.05$

and limonene, reduced cabbage root fly oviposition (GLMM, Poisson data, analysis of contrasts:  $P<0.01$  for each comparison with control) while only one VOC, HA, enhanced egg laying on broccoli plants (GLMM, Poisson data, analysis of contrasts:  $\chi^2=11.81$ , 1 d.f.,  $P<0.01$ ). MeSA and carvone did not modify the

number of eggs laid by the cabbage root fly (GLMM, Poisson data, analysis of contrasts,

respectively:  $\chi^2=0.65$ , 1 d.f.,  $P=0.46$  and  $\chi^2=0.79$ , 1 d.f.,  $P=0.44$ ). Infestation levels in the plots exceeded the threshold used by farmers to decide whether or not to spray against the pest (1 egg.day<sup>-1</sup>.plant<sup>-1</sup>) in the control plot (Wilcoxon rank sum test:  $V=834$ ,  $P<0.01$ ) and in three treatments: HA, MeSA and carvone (Wilcoxon rank sum test:  $P\leq 0.03$  for the three volatile tested, Figure 27).



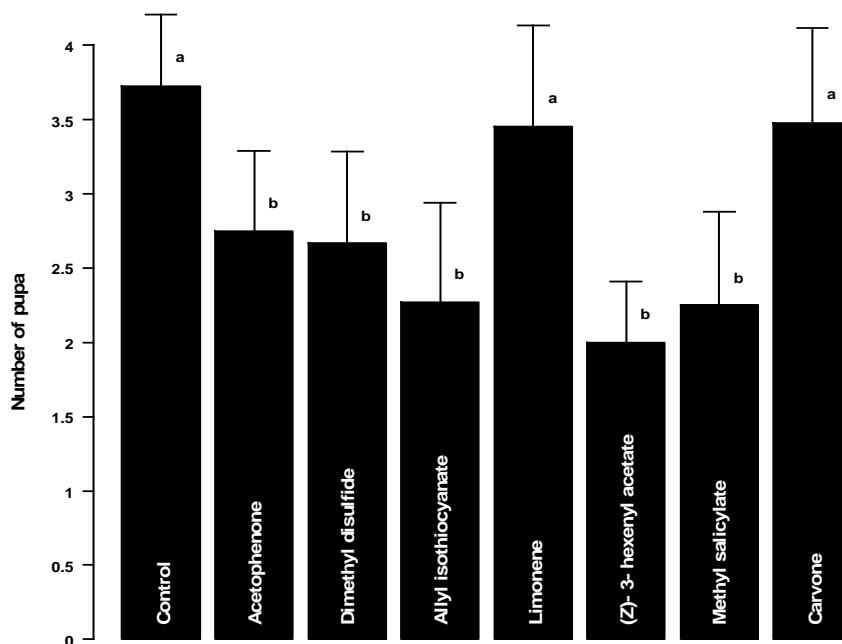
**Tableau 5** : Mean ( $\pm$  SE) cumulative number of *Delia radicum* eggs per plant and per distance from the dispensers

	0.50m	1m50	2m50
<b>Control</b>	28.63 $\pm$ 4.34 a A	42.19 $\pm$ 6.73 b A	42.00 $\pm$ 4.87 b A
<b>Acetophenone</b>	26.63 $\pm$ 5.67 ab A	32.38 $\pm$ 4.17 a B	24.50 $\pm$ 4.88 b B
<b>Dimethyl disulfide</b>	18.63 $\pm$ 3.47 a B	31.75 $\pm$ 5.13 b B	35.14 $\pm$ 6.46 b B
<b>Allyl isothiocyanate</b>	27.75 $\pm$ 4.91 ns A	31.13 $\pm$ 3.19 ns B	31.38 $\pm$ 6.40 ns B
<b>Limonene</b>	32.75 $\pm$ 4.51 ns A	35.88 $\pm$ 7.72 ns B	30.50 $\pm$ 6.10 ns B
<b>(Z)- 3- hexenyl acetate</b>	31.38 $\pm$ 3.62 a A	32.00 $\pm$ 4.28 a B	65.63 $\pm$ 11.97 b B
<b>Methyl salicylate</b>	40.57 $\pm$ 6.68 a B	41.88 $\pm$ 7.73 a A	33.88 $\pm$ 6.27 b B
<b>Carvone</b>	27.75 $\pm$ 4.33 a A	43.25 $\pm$ 7.52 b A	37.75 $\pm$ 6.42 b A

Means with different letters are significantly different: GLMM 'poisson', analysis of contrast and p.value correction (false discovery rate),  $P < 0.05$ . Small letters compare the effect of the distance for a same synthetic VOC. Capital letters compare the differences between the control and the synthetic VOC for a same distance

Within plots, cabbage root fly oviposition was significantly influenced by the distance from the dispensers (GLMM, Poisson data, likelihood ratio test:  $\chi^2=95.11$ , 2 d.f.,  $P < 0.001$ ) and the interaction with the volatile released (GLMM, Poisson data, likelihood ratio test:  $\chi^2=180.44$ , 14 d.f.,  $P < 0.001$ ). In the control plot, plants at the center of the plot received less eggs than plants positioned at both 1m50 and 2m50 (Table 5). A similar pattern was observed when dispensers of carvone or DMDS were present in the plots. In comparison to the control, DMDS reduced oviposition at each distance sampled while carvone did not affect the number of eggs. The release of MeSA reversed this pattern and more eggs were laid at both 0.50m and 1m50 than at 2m50. In comparison to the control, dispensers of HA did not modify plant infestation at 0.50m but oviposition was reduced at 1m50 while it was increased by over 57% at 2m50. In plots with AITC, limonene, and to a fewer extent ACPH, plants were as much infested in the center of plots as in the periphery.

**Pupae collection and emergences** Overall, we observed large differences between the number of eggs laid and the number of pupae collected. About 10% of eggs laid gave a pupae in the control plot. The different synthetic volatiles released had a significant effect on the number of pupae collected (GLMM, Poisson data, likelihood ratio test:  $\chi^2=25.08$ , 7 d.f.,  $P < 0.001$ ) (Figure 28). The presence of two synthetic volatiles, limonene and carvone, did not affect the number of pupae found on sampled plants while it was significantly reduced when the other compounds were released (GLMM, Poisson data, analysis of contrasts:  $P \leq 0.05$  for each comparison to the control). Within the experimental plots, the distance was only just



**Figure 28 :** Mean ( $\pm$  SE) number of *Delia radicum* pupa per plant over the season  
Means with different letters are significantly different: GLMM 'poisson', analysis of contrast and p.value correction (false discovery rate),  $P < 0.05$ .

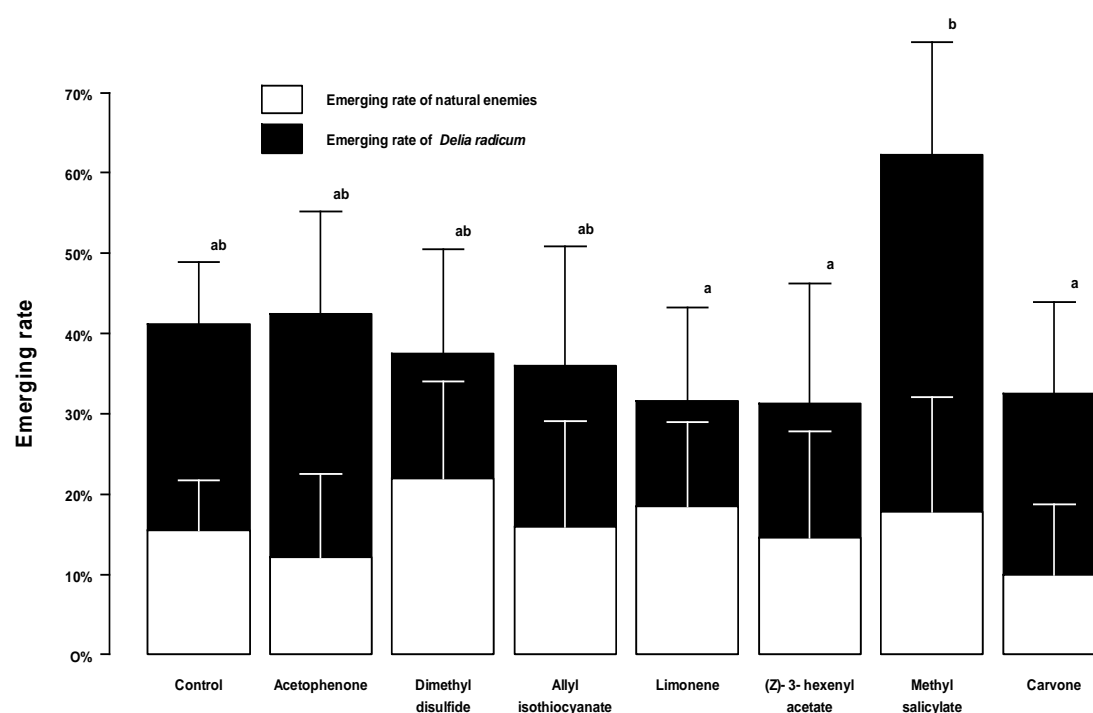
**Tableau 6 :** Mean ( $\pm$  SE) number of *Delia radicum* pupa per plant and per distance from the dispensers

	0,50m	1m50	2m50
<b>Control</b>	3.75 $\pm$ 0.87 ns A	4.13 $\pm$ 0.90 ns A	3.27 $\pm$ 0.78 ns A
<b>Acetophenone</b>	3.13 $\pm$ 0.95 ns A	3.13 $\pm$ 1.09 ns A	2.00 $\pm$ 0.80 ns A
<b>Dimethyl disulfide</b>	4.00 $\pm$ 1.60 a A	1.38 $\pm$ 0.46 b B	2.63 $\pm$ 0.71 ab A
<b>Allyl isothiocyanate</b>	1.58 $\pm$ 0.65 ns B	2.29 $\pm$ 1.17 ns A	2.88 $\pm$ 1.49 ns A
<b>Limonene</b>	4.83 $\pm$ 1.17 ns A	2.50 $\pm$ 0.68 ns A	3.38 $\pm$ 1.52 ns A
<b>(Z)- 3- hexenyl acetate</b>	1.50 $\pm$ 0.53 ns B	2.13 $\pm$ 0.64 ns B	2.38 $\pm$ 0.94 ns A
<b>Methyl salicylate</b>	3.86 $\pm$ 1.50 a A	1.83 $\pm$ 0.60 b B	1.00 $\pm$ 0.58 b B
<b>Carvone</b>	3.38 $\pm$ 1.46 ns A	4.29 $\pm$ 0.75 ns A	2.88 $\pm$ 1.01 ns A

Means with different letters are significantly different: GLMM 'poisson', analysis of contrast and p.value correction (false discovery rate),  $P < 0.05$ . Small letters compare the effect of the distance for a same synthetic VOC. Capital letters compare the differences between the control and the synthetic VOC for a same distance.

significant (GLMM, Poisson data, likelihood ratio test:  $\chi^2=5.96$ , 2 d.f.,  $P=0.05$ ) but the interaction with ‘volatile tested’ was stronger (GLMM, Poisson data, likelihood ratio test:  $\chi^2=34.77$ , 14 d.f.,  $P<0.01$ ) (Table 6).

The proportions of adults emerging from the pupae were relatively low and varied from 31% (HA) to 62% (MeSA) (Figure 29). These proportions differed slightly with treatments ( $\chi^2 = 15.52$ , 7 d.f.,  $P<0.05$ ). Such differences were only due to differences in cabbage root fly emergences ( $\chi^2 = 21.61$ , 7 d.f.,  $P<0.05$ ) as emergences of natural enemies did not differ ( $\chi^2 = 5.10$ , 7 d.f.,  $P=0.65$ ). Due to the low number of adults recovered, analysis about distances was not conducted.



**Figure 29 :** Proportions ( $\pm$  CI,  $\alpha = 0.05$ ) of adults emerging from pupae collected in the field. Natural enemies include *Trybliographa rapae* and *Aleochara bipustulata* and *A. bilineata*. Proportions with different letters are significantly different: pairwise  $\chi^2$  test with p.values correction (false discovery rate),  $P\leq 0.05$

## DISCUSSION

Our results indicate that the ecological function of VOCs can be exploited in field conditions to develop alternative crop protection. At the plot scale, although two compounds did not affect *D. radicum* oviposition (MeSA and carvone), four VOCs significantly reduced plant colonization (ACPH, DMDS, AITC and limonene) while one VOC increased it (HA). At the end of the season, the pupae collection indicates that all the compounds tested, except limonene and carvone, reduced herbivore pressures. The potential of DMDS and HA for developing a ‘push-pull’ strategy against *D. radicum* is consistent with previous conclusions from field experiments (Kergunteuil *et al.*, 2012). While DMDS could be used in a ‘push’ component to repel/deter cabbage root flies, HA could be used in a ‘pull’ component to simultaneously attract/stimulate *D. radicum* oviposition and limit herbivore development. However, our investigations on the effect of synthetic VOCs along a small spatial transect in the field outline challenges that will need to be resolved before actual applications.

At the plot scale, four VOCs reduced cabbage root fly oviposition (ACPH, DMDS, AITC and limonene). To our knowledge, this field study is the first to report an influence of limonene on *D. radicum* oviposition. The results obtained in the present study about the negative effect of ACPH do not match results of previous experiments where no influence of ACPH emission was observed (Kergunteuil *et al.*, 2012) suggesting that context-dependant responses are possible (Kaplan, 2012 b). However, we have also recorded stability in some VOC effects across years. DMDS was previously described as a stimulus limiting the number of eggs laid by *D. radicum* (Kergunteuil *et al.*, 2012) certainly due to its post-alighting role in patch quality assessment (Ferry *et al.*, 2009). Indeed, VOCs could act synergistically with chemocontact stimuli detected after landing and influence the host plant acceptance of flies (de Jong & Städler, 1999). VOCs are also used while insects are still in flight and AITC was early demonstrated as a long-range attractant for *D. radicum* (Finch & Skinner, 1982). Surprisingly, in the present study AITC dispensers did not increase oviposition of flies and even reduced it. The only compound found to increase the number of eggs laid was HA, a VOC involved in the attraction of other herbivores specialized on brassicaeous plants such as *Plutella xylostella* (Reddy & Guerrero, 2000; Dai *et al.*, 2008). Laboratory bioassays are required to understand how HA influences *D. radicum* behavior, either by long-range attraction or post-alighting stimulation. Overall, four VOCs reduced oviposition (ACPH, DMDS, AITC and limonene) while only one enhanced plant colonization (HA) indicating an

unbalanced ratio of negative and positive stimuli among the VOCs tested. During host plant finding behavior, olfactory signal recognition by herbivorous insects relies on the detection of a blend of VOCs ranging between three to ten compounds with particular proportions (Bruce *et al.*, 2005; Bruce & Pickett, 2011). Thus, high releases rates of additional synthetic VOCs may mask or disrupt natural signals emitted by host plants (either qualitatively or quantitatively) and consequently reduce the efficiency of host plant foraging by *D. radicum*. In their study, Brassch & Kaplan, 2012 also reported a general repellent effect of VOCs released for the different herbivores sampled. Although such hypotheses may explain reduced oviposition, other mechanisms could be involved in the positive influence of HA on cabbage root fly oviposition. HA has been shown to be involved in plant priming and enhance VOCs emissions even in intact plants (Engelberth *et al.*, 2004). Despite an initial defensive role, in some cases, such induction has been reported to increase plant apparency for herbivores (Halitschke *et al.*, 2008). Whether or not defense induction is involved in the observed effect, our results concur with previous field studies demonstrating an increase of herbivores on corn plants exposed to HA (von Mérey *et al.*, 2011).

The VOCs tested differentially affect cabbage root fly oviposition within plots depending on distance from the dispensers. In the control plot, we observed an edge effect: plants at the periphery received more eggs than central plants suggesting that peripheral plants were more visited by *D. radicum*. This colonization pattern was conserved when dispensers of carvone and DMDS were placed in the plot. Although the former did not modify the number of eggs laid, DMDS reduced oviposition at each distance sampled. The intensity of egg laying reduction remains clearly pronounced at 2m50 suggesting that DMDS influences cabbage root fly behavior over the spatial scale studied. Considering that DMDS serves in patch quality assessment, this VOC may be involved in functional decision such as oviposition once flies have landed (Ferry *et al.*, 2009). In our experiment, the high release rate of DMDS compared to natural emission rates (Ferry *et al.*, 2007) could explain the influence of this VOC on *D. radicum* oviposition over a distance of several meters from the point-source.

Although MeSA did not modify *D. radicum* oviposition at the plot scale, assessment of its effect on egg laying depending on distance from dispenser revealed significant differences. Inside plots with MeSA dispensers, more eggs were laid at both 0.50m and 1.50m than at 2.50m. Thus, the colonization pattern seemed reversed compared to control plots. The contrasts observed between colonization patterns of plots exposed to MeSA and control plots

suggest that MeSA attracts cabbage root flies near the dispensers annulling the edge effect. Investigations about MeSA largely concerned natural enemy attraction (James & Price, 2004) but this VOC is also involved in the attraction of various herbivores such as *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), *Spodoptera eridania* (Lepidoptera: Noctuidae) and *Diaphania hyalinata* (Lepidoptera: Pyralidae) (Dickens, 2000; Meagher & Landolt, 2008). The limited area where MeSA increased oviposition is in agreement with previous studies which have outlined the local concentration of insects responding positively to MeSA, *i.e.* at a distance  $\leq 1.5$  m from a point-source (Lee, 2010; Mallinger *et al.*, 2011; Rodriguez-Saona *et al.*, 2011). The two contrasted repartition patterns observed in control plots and plots with MeSA dispensers fit well with the redistribution hypothesis proposed by Braasch & Kaplan, 2012. MeSA may attract *D. radicum* near the dispensers (0.50m and 1m50) and consequently reduce insect density in adjacent zones (2m50). However, we cannot exclude that this pattern of attraction may be more complex than simple directional responses. In addition to plant-insect communication, MeSA is also known to play a critical role in plant-plant communication. This VOC can directly affect plant physiology and consequently modify plant acceptance by insects. Thus, it appears difficult to link the behaviour observed with a particular effect of MeSA on broccoli plants at this stage (James & Price, 2004).

However, optimal concentrations for insects responding to the VOCs tested may not necessarily be the ones found close to dispensers. The comparison of control and HA plots revealed a surprising spatial pattern : the numbers of eggs were not different at 0.50m but HA reduced oviposition at 1m50 while it increased the number of eggs by 57% at 2m50. Hence, we can hypothesize that the HA concentration was most attractive at 2.50m where the highest number of eggs was observed. This attraction at 2.50m resulted in a depletion of the pest in adjacent areas, at 1.50m, while the 0.50m zone was unaffected compared to control. Further studies would be necessary to test the redistribution hypothesis using sticky traps to distinguish dynamic effects from functional influences of VOCs on *D. radicum* oviposition. Nevertheless, a general increase of oviposition is recorded in plots exposed to HA. Although this VOC remains interesting for the ‘pull’ component, the redistribution of flies in the plot raises important questions about the deployment of HA. Comparing the effects of MeSA and HA, the former concentrates *D. radicum* not as far from the dispenser as HA. However, MeSA presents a relatively broad scale of attractiveness whiles an attractive concentration of HA results in a depletion of the cabbage root fly in the adjacent area. When AITC, limonene, and in a fewer extent ACPH, were released in the plots the number of eggs did not differ

across the distances sampled. These three VOCs cancelled the edge effect observed in the control plot by reducing the number of eggs laid at 1m50 and 2m50 without altering *D. radicum* oviposition at 0.50m. Reasons for this are unknown and further studies are required to understand more precisely underlying mechanisms.

Predictably, the reduction of eggs laid in plots exposed to ACPH, DMDS, and AITC resulted in a lower number of pupae. However, synthetic release of MeSA and HA also reduced the number of pupae despite their null or positive influence on oviposition indicating that these two VOCs alter cabbage root fly development. These contrasts could rely on plant defence induction or recruitment of natural enemies.

In control plots, contrary to what was observed for oviposition, distance did not affect number of pupae collected. These results could be due to a higher density of egg predators in the border than in the middle of the plots. Important ground-dwelling egg predators of the cabbage root fly such as *Bembidion lampros* prefer bare ground habitat to plant shelter when they are not searching for prey (Mitchell, 1963). In our field setup, we can hypothesize that egg predators spent much time in the bare ground between the experimental plots and that their control on *D. radicum* occurred mostly in the border of the plots, thereby levelling off number of pupae found across the three distances sampled. The role of MeSA in the recruitment of natural enemies has been extensively demonstrated (Rodriguez-Saona *et al.*, 2011). The limited number of pupae collected in plots with MeSA dispensers may be due to a stimulation of egg predation activity as shown previously (Kergunteuil *et al.*, 2012). The number of pupae was reduced at both 2m50 and 1m50 but central plants were as much infested as in control plots supporting the edge effect hypothesized above for egg predators. Also, plots exposed to HA presented a smaller number of pupae than control plots but the reduction was this time observed on central plants and at 1.50m. Although induction of plant defences by HA could enhance plant apparency to adult flies as previously suggested, induced plants could also present higher resistance to larval development. A similar paradox was already shown for broccoli plants induced by salicylic acid: plants induced by this phytohormone tended to harbour more eggs but significantly reduced adult emergences (Pierre *et al.*, 2013). Carvone influenced neither oviposition nor number of pupae. However, plants exposed to limonene exhibited fewer eggs than control plants but as many pupae suggesting that this VOC could alter foraging efficiency of egg predators.

Differences in emerging rates across the different treatments remained very small. At the plot scale, the emergence of natural enemies did not differ suggesting that VOCs tested did not affect host foraging efficiency of parasitoids. The contrasts about general emerging rates were principally due to the higher number of flies emerging from plants exposed to MeSA. Due to the low number of emergent obtained it appears difficult to conclude precisely about the influence of the VOCs tested on emerging rates.

Despite the relatively small spatial scale studied here, oviposition contrasts among the three distance sampled bring important information about the use of VOCs in a ‘push-pull’ strategy against *D. radicum*. DMDS reduces oviposition over a large area surrounding the dispenser and seems to be a good candidate for the ‘push’ component. The two compounds locally increasing plant infestation, MeSA and HA, are active at different distances. However, both compounds also decrease plant infestation at one of the distances sampled, possibly limiting the overall benefit of the ‘pull’ component. Further studies based on a combination of these two VOCs in a ‘pull’ component would be required to investigate possible synergistic effects. The assessment of the influence of VOCs at distances larger than 2m50 is necessary for future applications and would be useful to confirm the redistribution hypothesis. Nevertheless, the use of HA and MeSA in the ‘pull’ component are promising as they locally increase oviposition but reduce number of pupae at the plot scale. The results obtained in the present study are partially consistent with our previous experiments conducted in 2011 and reinforce the selection of DMDS and HA for a ‘push-pull’ strategy. However, we also observe differences across the two years despite comparable release rates demonstrating context-dependent responses (Kaplan, 2012 b). The present study highlights the fact that pest management based on synthetic VOCs is complex and involves mechanisms not yet well understood.

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## APPENDIX

### Diffusion rate per dispenser (mg.day<sup>-1</sup> ± SEM)

	Week 1	Week 2	Week 3	Week 4
<b>Carvone</b>	5.09 ± 1.13	4.28 ± 0.79	7.12 ± 0.63	3.91 ± 0.47
<b>Acetophenone</b>	16.42 ± 1.61	9.11 ± 0.50	14.10 ± 1.38	5.87 ± 0.55
<b>Methyl salicylate</b>	16.07 ± 2.26	7.31 ± 1.22	20.07 ± 2.00	6.13 ± 0.94
<b>(Z)-3-hexenyl acetate</b>	45.53 ± 4.49	41.03 ± 4.19	59.85 ± 4.86	32.50 ± 1.82
<b>Allyl isothiocyanate</b>	60.02 ± 3.43	58.93 ± 2.06	55.85 ± 6.57	37.94 ± 1.94
<b>Limonene</b>	64.24 ± 5.57	45.68 ± 4.17	55.71 ± 3.52	45.25 ± 4.12
<b>Dimethyl disulfide</b>	170.05 ± 5.81	269.40 ± 8.43	203.85 ± 4.80	144.93 ± 4.37

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**ARTICLE N°5 : Des ceintures végétales  
pour protéger les cultures de brocoli  
contre *Delia radicum*.**

*Article en préparation*





# Potential of brassicaceous plant belts for the protection of broccoli crops against the cabbage root fly: a field study

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## ABSTRACT

A field study was conducted in order to study the influence of different plant belts on the level of *Delia radicum* infestation on broccoli crop. Two potentially attractive belts were tested using turnip (*Brassica rapa*) and Chinese cabbage (*B. rapa pekinensis*). These two plants species have previously been shown to attract and/or stimulate egg laying in *D. radicum*. In addition we also examined the effect of a potentially dissuasive belt planted with white mustard (*Sinapis alba*), a plant known to harbour low levels of infestation by *D. radicum*. Our results indicate that the only plant belt reducing broccoli infestations was the one planted with Chinese cabbage: the number of eggs laid on central broccoli plants was reduced by 20% and the number of pupae collected on broccoli decreased by over 45%. In addition of limiting egg-laying on adjacent broccoli plants, Chinese cabbage could also enhance *D. radicum* mortality through either top down (*i.e.* predation) or bottom up (*i.e.* altered larval development) mechanisms that still need to be elucidated. Because of this effect on pest survival, Chinese cabbage could be used as a “dead-end” trap crop around broccoli fields.

**Key words:** *Delia radicum*, trap crop, dissuasive belt, broccoli, white mustard, turnip, Chinese cabbage.

# INTRODUCTION

The combination of different plants for pest control is certainly not a new topic. In records dating back to antiquity, Pliny the Younger described how it was possible to reduce pest density on cruciferous plants associated with the common vetch, *Vicia sativa* (Schoonhoven *et al.*, 1998 cited by Finch & Collier, 2001). Strategies based on plant diversification present two major advantages that can explain their actual interest in crop protection. Indeed, plant diversification represents an alternative pest control strategy that limits environmental costs of pesticides and remains safe for human health. Ecological engineering based on habitat manipulation with natural sources offers choice situation to insects and consequently increases durability of this pest control strategy (Gurr *et al.*, 2004).

Two main strategies based on plant diversification can be used to limit crop losses. First, repellent and/or dissuasive plants can be intercropped in the commercial field (Andow, 1991). Second, attractive and/or stimulant plants, can be planted in zones adjacent to the target crop, in order to retain pests into these zones (Shelton & Badenes-Perez, 2006). This so-called “trap crop” strategy has been used to protect commercial fields against various pests such as parasitic plants, nematodes and insects (Hokkanen, 1991). The pest concentration in restricted areas enhances pest control that can be achieved either through biological control, insecticide applications or mechanically (Cook *et al.*, 2007, Swezey *et al.*, 2007). Although only 10 cases of trap crops have been successfully used so far, research on this topic is still active and concerns a large number of phytophagous insects (Shelton & Badenes-Perez, 2006). Overall, three different trap crop strategies can be distinguished. Conventional trap crops involve naturally more attractive/stimulant plants as food sources or oviposition sites (Swezey *et al.*, 2007). Dead-end trap crops consist of plants that are attractive to pests but simultaneously limit (or impeded) their development (Shelton & Nault, 2004). In assisted trap crops, additional stimuli such as pheromones, plant volatiles or food supplements are used to reinforce the efficiency of one of the two previous trap cropping strategies (Martel *et al.*, 2005).

The purpose of the present study is to select plants that could be used to protect broccoli (*Brassica oleraceae*) crops against the cabbage root fly, *Delia radicum*. Females of this important pest lay eggs in the soil close to plant stem and larva cause agronomic damages by feeding on the roots. In an agronomic context, ground dwelling egg predators including

carabidae and staphylinidae are particularly important for natural control of the fly (Coaker & Williams, 1963; Prasad & Snyder, 2004). The recent limitation of insecticides toward *D. radicum* in Europe requires the development of alternative crop protection methods and trap cropping appears like a promising approach. Indeed, our previous field studies have revealed important differences in the preference of this pest among different plant species in its host range (Kergunteuil *et al.*, in prep).

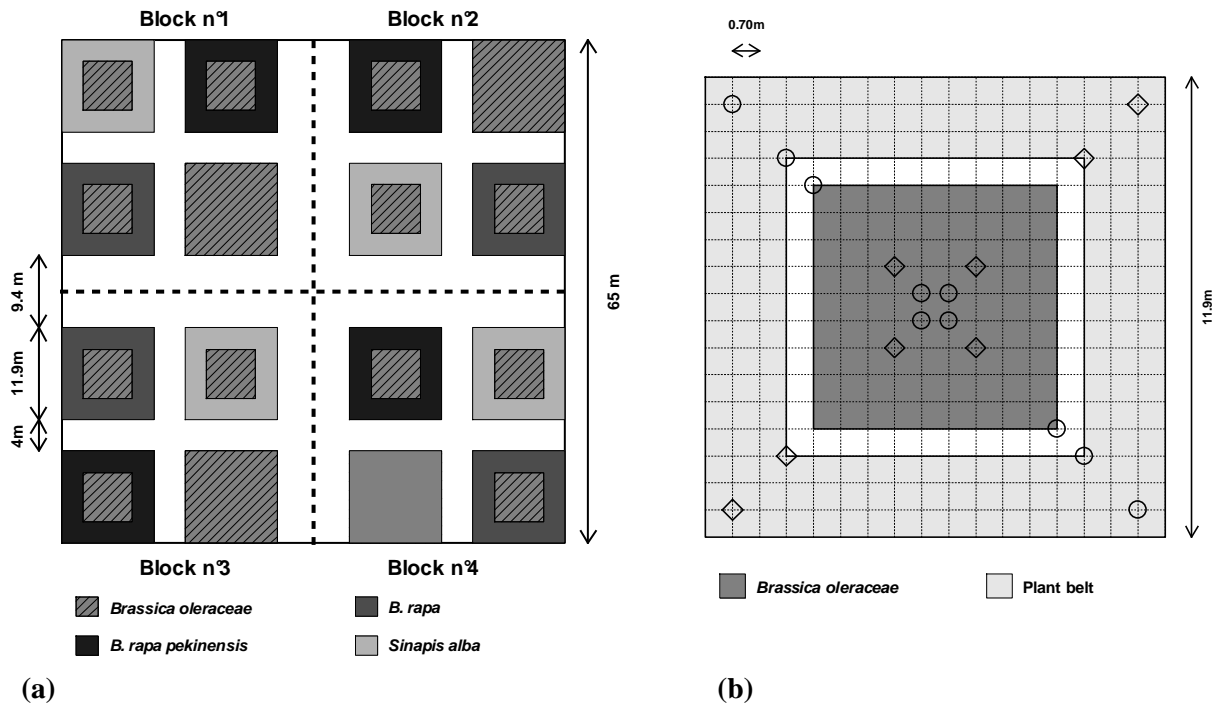
In a field experiment, we surrounded broccoli plots with two distinct plant belt types. For the first type of belts, we selected two plants previously described in the literature as attractive or stimulant for *D. radicum*: turnip, *Brassica rapa* (Doane & Chapman, 1962) and Chinese cabbage, *Brassica rapa pekinensis* (Rousse *et al.*, 2003). The use of these two brassicaceous plants could therefore be used in a conventional approach where trap crops concentrate phytophagous insects. For the second type, we selected white mustard, *Sinapis alba*, a plant with low level of infestation by the cabbage root fly in the field (Kergunteuil *et al.*, in prep). The use of such plant could be quite original as it could serve as a deterrent belt. We assessed the influence of these three types of plant belts on both plant colonization and plant infestation at the end of the growing season. Our results are discussed in relation to previous work about the development of a “Push-Pull” strategy against *D. radicum*.

## MATERIALS AND METHODS

### Field experiment

**Field setup** The field experiment was performed during spring 2012 and took place at the experimental station of “La Motte” (INRA Center), Le Rheu, Brittany, France (48°07'15'' N, 1°47'39'' W). Plants were provided by a commercial provider (Thomas Plants, Ploubazlanec, France): seeds were sown individually in peat soil cylinders and grown for 6 weeks in a plastic tunnel before delivery, as requested no pesticide were used. Seedlings were transplanted in the field on April 12<sup>th</sup>. The field was cultivated according to usual agricultural practices and no pesticides were applied. We used a randomized block design consisting of 4 blocks (1056m<sup>2</sup>) with 4 experimental plots (18x18 plants, 142m<sup>2</sup>) in each block (Fig. 30).

Blocks were separated from each other by 9.4m of bare soil while plots in the blocks were separated by 4m. In each block, one control plot was planted with broccoli (*Brassica oleraceae*) only and three treatment plots were planted with broccoli (10x10 plants) surrounded by different plant belts (4 rows): turnip (*B. rapa*), Chinese cabbage (*B. rapa pekinensis*) or white mustard (*Sinapis alba*).



**Figure 30.** Experimental field setup

(a) Schematic representation of the experimental field. It consisted of 4 blocks as repetitions, each containing one control plot planted with broccoli (*Brassica oleraceae*) and three treatments plots where broccoli was surrounded by different belts planted either with turnip (*B. rapa*), Chinese cabbage (*B. rapa pekinensis*) or white mustard (*Sinapis alba*). (b) A plot comprised 18x18 plants distributed on a 0.70x0.70m grid. Oviposition of *Delia radicum* was monitored with felt traps (open circle) placed on 6 plants into the middle of the plots and 4 plants into the plants belts. Plants used for pupa collection (diamonds) included 4 plants into the middle of the plots and 4 plants into the plants belts

### Cabbage root fly oviposition

Egg laying by *D. radicum* was monitored using felt traps (for a description, see Bligaard *et al.*, 1999) positioned around the stem of the plants, where flies deposit their eggs. In each plot, felt traps were placed on six plants in the middle/center of the plot and four plants in the plant belts (Fig. 30). Traps were collected every week and the eggs found inside were counted and removed. Emptied traps were then replaced on the same plants (or on the nearest plant when this plant had died). Cabbage root fly infestation was monitored from the 3<sup>rd</sup> to the 24<sup>th</sup> of May.

**Pupae collection and emergences** Pupae were collected in the field on June 18<sup>th</sup>, *i.e.* four weeks after the end of the oviposition period of *D. radicum*, thereby allowing a complete larval development of the eggs present. In each plot, pupae were sampled on four plants in the middle of the plot and four plants in the plant belts (Fig. 30). Approximately two liters of soil (15-20 cm deep, 20cm of diameter from the stem) from around the roots of selected plants was collected, stored in a cold chamber (4°C) and maintained in the dark until the collection of pupae. Each sample was washed in water and pupae were separated from the soil by flotation.

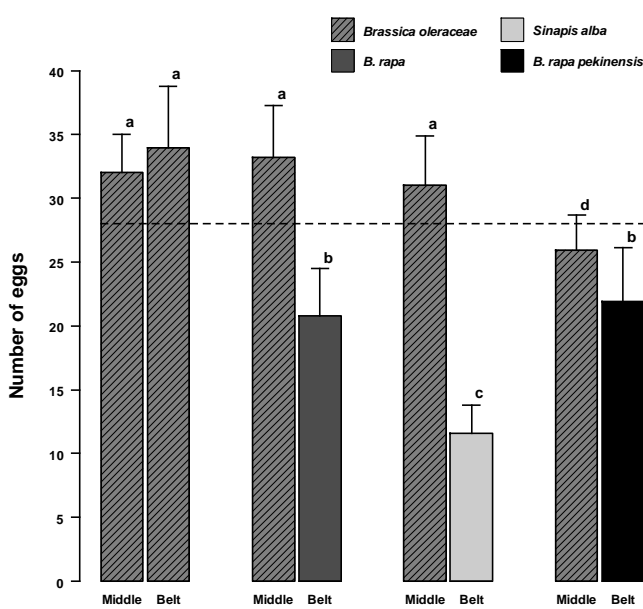
## Statistical analyses

All statistical analyses were carried out with R software (R development core team, R version 2.12.1). Analyses about cabbage root fly oviposition were conducted on the cumulative number of eggs laid per plant over the four sampling weeks. The number of *D. radicum* eggs laid and the pupae found per plant were analyzed using two generalized linear mixed models (GLMM, package 'lme4') for Poisson data (linking function: 'log') with spatial 'block' included as random factor. Comparisons between treatments were carried out using an analysis of contrast (package 'doBy') and p.values were adjusted by a false discovery rate correction method.

## RESULTS

The number of eggs laid in the middle of each plot (*i.e.* broccoli) varied between the four treatments (GLMM for Poisson data, likelihood ratio test:  $\chi^2 = 23.86$ , 3 d.f.,  $P < 0.001$ ). Although the number of eggs laid on broccoli was unaffected on plants surrounded by turnip and white mustard, belts planted with Chinese cabbage significantly reduced broccoli colonization in the middle of the plot (Figure 31). Chinese cabbage lowered the number of eggs laid on broccoli under the threshold used by farmer to decide whether or not to spray against the pest. Overall, broccoli was the plant harboring the highest number of eggs while the three other plants exhibited lower levels of eggs. The lower number of eggs laid on these three plants (turnip, white mustard or Chinese cabbage) resulted in a lower number of pupae on these plants (Figure 32). The only plant belt reducing broccoli infestation was the one

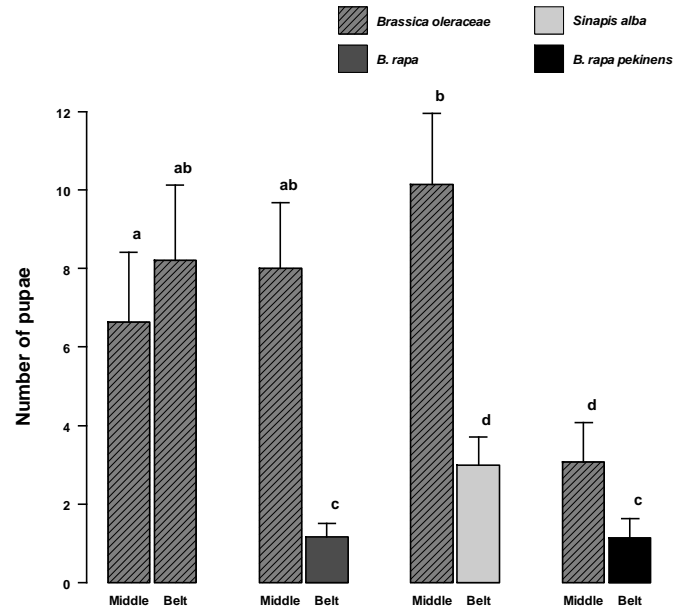
planted with Chinese cabbage. Differences in plant infestation were more pronounced than the ones observed in plant colonization.



**Figure 31.** Mean ( $\pm$  SE) cumulative number of *Delia radicum* eggs per plant

Number of eggs found per felt trap was cumulated on four sampling dates (3, 10, 17, 24 May). The dashed line corresponds to the threshold of 28 eggs per felt trap per month, which is habitually used by farmers to decide whether or not to spray against the pest.

Means with different letters are significantly different: GLMM 'poisson', analysis of contrast and p.value correction (false discovery rate),  $P < 0.05$ .



**Figure 32.** Mean ( $\pm$  SE) number of *Delia radicum* pupae per plant

Means with different letters are significantly different: GLMM 'poisson', analysis of contrast and p.value correction (false discovery rate),  $P < 0.05$ .

## DISCUSSION

The present study indicates that both plant colonization and plant infestation of broccoli plots can be influenced by the type of plant belt planted around these plots. Hereafter, the effects of the different belts are discussed plant by plant.

The white mustard, *Sinapis alba*, is known to exhibit low levels of *Delia radicum* infestation (Dosedall *et al.*, 1994; Kergunteuil *et al.*, in prep). So far, the majority of studies

about *S. alba* concerned the identification of resistant plants that could be used by breeders in order to develop new brassicaceous cultivars (Dosdall *et al.*, 2000). Our results confirm that the cabbage root fly lays few eggs on the white mustard in comparison to other brassicaceous plants but indicates that such resistant traits are inefficient to develop habitat management strategies based on plant belts using this species. Indeed, broccoli plant surrounded by *S. alba* were as much infested as the broccoli planted in the middle of the control plot. Thus, we can hypothesize that belts consisting of dissuasive plants do not represent physical barriers impeding plant colonization by the cabbage root fly. Further experiments are required to examine alternative modalities of white mustard deployment (*e.g.* intercropping) in order to decide whether or not *S. alba* could be used to protect broccoli crops.

Several studies have shown that turnip, *Brassica rapa*, can be highly infested by the cabbage root fly (*e.g.* Doane & Chapman, 1962). It has even been already proposed as a possible trap crop to protect broccoli crops against *D. radicum* (Rousse *et al.*, 2003). However, turnip plants were poorly colonized in the present study and did not reduce the cabbage root fly infestation on the associated broccoli plants. This field experiment confirms previous results conducted in the field where we have shown that *B. rapa* exhibits high attack rate of the herbivore guild locally presents (*Plutella xylostella*, *Pieris rapa*, *Phyllotetra* spp.) (Kergunteuil *et al.*, in prep). This high level of infestation by other herbivores may have altered the quality of the plant for the cabbage root fly and could be responsible for the low infestation levels observed. Thus, our initial hypothesis about the potential of turnip in plant protection could not be confirmed here. In addition to the observation of *D. radicum* behaviour, our results highlight the necessity to consider the presence of other herbivore species in future investigations.

The only belt allowing some protection of broccoli against the cabbage root fly was the one planted with Chinese cabbage, *Brassica rapa pekinensis*, a plant previously described as highly attractive toward *D. radicum* (Rousse *et al.*, 2003). In our field study, the presence of Chinese cabbage areas around broccoli plots reduced by 20% the number of eggs laid on central broccoli plants. According to the classical hypothesis about “trap crop” mechanisms, the efficiency of *B. rapa pekinensis* may rely on an increase of *D. radicum* oviposition on this plant although we could not observe such effect here. This paradox could result from an experimental bias conducting to underestimate the number of eggs laid on this plant. Chinese cabbage displays a complex architecture in comparison to the other brassicaceous plants



tested. While the assessment of *D. radicum* oviposition with felt traps was relatively accurate on broccoli, white mustard and turnip due to a long stem without any leaves, the use these traps may not be adapted on Chinese cabbage which have a reduced stem and basal leaves. An additional field experiment where fly oviposition was assessed directly on leaf insertion (unpublished data) confirmed an experimental bias in the present study. Even so, our results indicate that belts planted with Chinese cabbage could be efficient to limit number of eggs laid on central broccoli plants. More importantly, this belt also reduced by 46% the number of pupae recovered on broccoli plants suggesting that central plants are less exposed to herbivore pressure when they are surrounded by Chinese cabbage. Interestingly, the beneficial influence of Chinese cabbage on broccoli protection was more pronounced for number of pupae than number of eggs. In addition to plant colonization, the number of collected pupae results from both larval development (“bottom-up” control) and biocontrol by natural enemies (“top-down” control). In central plants of control plots and plots surrounded by Chinese cabbage, the pupae were collected on the same plants (*i.e.* broccoli) but placed in distinct habitats. Thus, the beneficial influence of Chinese cabbage on broccoli infestation could mainly rely on “top-down” control. Previous studies have shown that plant belts could also represent reservoirs for natural enemies and consequently enhance the efficiency of pest control (Shelton & Badenes-Perez, 2006). In this context, the low number of pupae collected from Chinese cabbage could result from attraction and retention of eggs predators of *D. radicum* such as *Metallina lampros*, *Aleochara* spp., *Bembidion* spp. Moreover, Chinese cabbage could represent unsuitable host plants for below-ground herbivores due its low root mass (Rousse *et al.*, 2003). The larval development of cabbage root maggots could be limited on such plants with poor nutritional resource; therefore the low number of pupae collected on Chinese cabbage may also rely on “bottom-up” mechanisms. Herbivore reduction makes the Chinese cabbage a good candidate for “dead-end” trap cropping. Hence, a belt planted with Chinese cabbage could be interesting to limit pest development in space (from the trap crop to the commercial field) and time (from one growing season to the next). Further studies concerning the potential of Chinese cabbage in cabbage root fly control should examine the potential influence of plant phenology and plant density in both the target field and the trap crop (Hokkanen, 1991; Musser *et al.*, 2005).

In addition to the present study, our research group is also investigating a parallel approach based on dispensers of synthetic VOCs for behavioral manipulation of *D. radicum*. In an applied perspective, we aim at designing a pest management method where trap crops

could be assisted by attractive VOC releases. In such approach pest control could be reinforced with deterrent VOC dispensers placed in the center of the field. In this “Push-Pull” strategy, the cabbage root fly could be repelled from target fields and redirected toward a VOC assisted trap crop where Chinese cabbage could serve as pest sink.

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# **DISCUSSION GENERALE**



La découverte il y a plus de vingt ans du rôle des composés volatils émis par les plantes attaquées dans le recrutement des ennemis naturels (Dicke & Sabelis, 1988 ; Turlings *et al.*, 1990) a ouvert la voie à de nombreuses recherches autour des interactions plantes - insectes (Mumm & Dicke, 2010). L'élégance du mécanisme permettant à une plante « d'appeler au secours » des « gardes du corps » à l'aide de signaux imperceptibles par l'Homme (et gardant donc une part de mystère) a sans doute contribué à ce succès. L'ensemble de ces recherches a mobilisé différentes disciplines écologiques (comportementale, évolutive et chimique) et a permis de faire un bond en avant dans notre compréhension des communications plantes-insectes. L'intérêt appliqué de ces résultats a très rapidement été mis en avant, bien qu'il ait fallu attendre près d'une dizaine d'années avant que de premières études ne soient menées en conditions naturelles. Au milieu des années 2000, l'équipe de David G. James a été la première à diffuser en plein champ des composés organiques volatils (COVs) de synthèse pour tenter de protéger des vignobles et des cultures de houblon en attirant des ennemis naturels généralistes (James, 2003 ; James & Price, 2004, James, 2005). A partir de résultats encourageants ayant démontré que les COVs synthétiques favorisent le recrutement d'auxiliaires de culture, les recherches se sont élargies à différents types de cultures au cours de ces dix dernières années (Khan *et al.*, 2008 ; Rodriguez-Saona *et al.*, 2011 ; Simpson *et al.*, 2011). Cependant, la grande majorité des études s'étant intéressées au potentiel des COVs dans la protection des cultures s'est restreinte au cadre de la lutte par contrôle biologique. Les stratégies de lutte de type « Push-Pull » constituent une nouvelle approche au sein de laquelle il devient possible de tirer profit de l'influence des COVs sur l'ensemble des niveaux d'un système tri-trophique où ils agissent (*cf.* introduction).

## **1. Synthèse des travaux et principaux résultats**

En partant du principe qu'une méthode de lutte ciblée doit commencer par s'intéresser au ravageur, nous avons cherché en priorité à modifier le comportement de l'herbivore. Les deux premiers articles de cette thèse ont permis de sélectionner des plantes et des COVs pouvant être utiles à la mise en place d'une méthode de lutte basée sur la modification comportementale d'un important ravageur des cultures de brassicacées, la mouche du chou *Delia radicum*. Les trois derniers articles ont permis d'étudier en plein champ la possibilité de



modifier la colonisation des plantes par la mouche du chou à l'aide de diffuseurs de COVs synthétiques ou de ceintures végétales au sein de stratégies « Push-Pull ». Après s'être concentré sur la modification comportementale du ravageur, les différents modes d'action des COVs à chacun des niveaux d'un réseau tri-trophique ont constitué la trame de fond à partir de laquelle nous avons discuté nos résultats de terrain.

Tous les insectes phytophages montrent des préférences au sein de leurs spectres d'hôtes. Dans un contexte agronomique, la mise en place de composantes végétales à partir de différents génotypes de plantes permet de modifier la répartition du ravageur au sein de la parcelle et de limiter les dégâts sur la culture d'intérêt : des plantes pièges servant à installer des zones attirant l'herbivore (Hokkanen, 1991 ; Shelton & Badenez-Peres, 2006) peuvent être combinées à des plantes le repoussant de la culture d'intérêt et conduire à des stratégies de type « Push-Pull » (Cook *et al.*, 2007). Dans **le premier article**, nous avons sélectionné en plein champ des plantes contrastées vis-à-vis de leurs taux d'infestation par *D. radicum*. Ces plantes représentent des candidats intéressants pour le développement de composantes « Push » ou « Pull » destinées à protéger les cultures de brocoli. Parmi cette première sélection de plantes candidates, certaines présentent un intérêt agronomique qui faciliterait leur insertion dans une méthode de protection des cultures. La moutarde blanche (*Sinapis alba*) limitant la ponte de *D. radicum* est par exemple cultivée pour ses graines, principal ingrédient des moutardes commercialisées, ou pour son feuillage en tant que fourrage ou engrais vert (Warwick, 2011). Parmi les autres plantes testées, certaines ne sont pas inscrites au catalogue des semences accessibles aux agriculteurs mais permettent d'ouvrir des axes de recherches prometteurs. C'est notamment le cas des deux génotypes de colza (*Brassica napus*) « Yudal et « Darmor-bzh » qui présentent des taux d'infestation fortement contrastés malgré leur appartenance à la même espèce. Ces deux génotypes ont récemment été utilisés pour étudier les liens entre diversité génétique et diversité métabolique (Wagner *et al.*, 2012). L'identification de régions génomiques impliquées dans l'émission de COVs modulant le comportement de *D. radicum* pourrait être utilisée par les sélectionneurs pour obtenir des cultivars intéressants à mettre en place dans une stratégie de type « Push-Pull ». En effet, des études olfactométriques nous ont permis de mettre en évidence le rôle des COVs émis par certaines de ces plantes, notamment pour ces deux génotypes de colza, dans leur interaction avec la mouche du chou : les degrés d'attractivité obtenus au laboratoire sont fortement corrélés avec les taux d'infestations observés sur le terrain. De manière générale, la sélection d'une plante hôte par un insecte herbivore repose sur une combinaison de signaux incluant

des stimuli gustatifs, visuels et olfactifs dont les importances relatives peuvent varier en fonction de caractéristiques spécifiques à l'herbivore (Schoonhoven *et al.*, 2005). Cette première étude confirme le rôle des stimuli olfactifs dans les prises de décisions comportementales de la mouche du chou et permet d'envisager l'utilisation de COVs pour protéger les cultures contre *D. radicum*.

Dans le **deuxième article**, nous avons élargi le nombre d'observations comportementales en olfactométrie et couplé cette première approche avec une analyse en chromatographie gazeuse des bouquets d'odeurs libérés par chacune des plantes testées. L'objectif de cette seconde étude était double. Nous souhaitons tout d'abord poursuivre la sélection de plantes répulsives ou attractives pouvant être utilisées dans les composantes « Push » ou « Pull ». La caractérisation des profils de COVs émis par des plantes plus ou moins attractives avait pour but d'identifier des composés volatils susceptibles de modifier le comportement de *D. radicum* et pouvant par la suite être diffusés au sein de parcelles expérimentales. Cette étude a permis de révéler que des plantes présentant des degrés d'attractivité contrastés émettaient des profils de COVs pouvant se différencier quantitativement et qualitativement. Les plantes les plus attractives émettent des quantités plus importantes de composés volatils et pourraient ainsi être plus faciles à localiser par l'herbivore dans un environnement complexe (Halitschke *et al.*, 2008). La visibilité d'une plante dans son environnement et le rôle joué par les COVs dans l'attraction des insectes herbivores peut, de ce point de vue, contribuer à définir le degré de résistance antixénotique présenté par la plante (Smith *et al.*, 2005). De plus, des différences qualitatives au sein des profils de COVs laissent penser que certains sesquiterpènes comme l' $\alpha$ -farnésène ou le  $\beta$ -caryophyllène sont impliqués dans l'attraction de la mouche du chou. Cette étude est la première à indiquer que ces deux COVs puissent être impliqués dans l'attraction à distance de *D. radicum* ; des études précédentes ont déjà montré leurs rôles dans l'attraction d'autres insectes herbivores comme *Cydia pomonella* et *Lobesia botrana* (Yan *et al.*, 2003; von Arx *et al.*, 2011). L'utilisation de COVs attractifs pourrait venir renforcer l'efficacité de ceintures pièges protégeant les cultures d'intérêt contre la mouche du chou et permettre de réduire la surface allouée à la composante « Pull » pour augmenter la surface de la culture à récolter.

Au cours de la thèse, nous avons mené de front plusieurs étapes dans nos recherches. Parallèlement aux deux travaux précédents (*cf.* article n°1 et 2), nous avons également étudié en conditions naturelles le potentiel de certains COVs synthétiques dans la protection des

cultures. **Le troisième article** se base ainsi sur une sélection bibliographique de composés volatils ayant préalablement été décrits comme pouvant influencer le comportement de la mouche du chou ou de ses ennemis naturels. Ce travail a d'abord permis de vérifier *in natura* la modification comportementale de *Delia radicum* et de ses ennemis naturels à l'aide de diffuseurs de COVs synthétiques placés au sein de parcelles expérimentales de brocoli (*B. oleraceae* var. *italica*). Nous avons montré que même si un COV comme l'acétophénone réduit l'efficacité des prédateurs d'œufs de la mouche du chou, un autre COV comme le salicylate de méthyle (MeSA) a tendance à favoriser le contrôle naturel de *D. radicum* par ses ennemis naturels. L'utilisation de COVs synthétiques a donné lieu à de nombreuses études ayant permis de montrer leur potentiel en contrôle biologique mais leur mode d'action reste encore à élucider. En effet, les stimuli olfactifs peuvent être utilisés par les ennemis naturels pour adapter à distance leurs comportements de recherche de nourriture ou influencer l'utilisation des ressources une fois qu'ils sont à proximité du site de nutrition (Mumm & Dicke, 2010 ; Hare, 2011). Alors que la majorité des études liées à la diffusion de COVs synthétiques se sont contentées de savoir dans quelle mesure il était possible d'attirer des auxiliaires de cultures, le succès d'une telle stratégie repose *in fine* sur leur activité au sein de la parcelle (Ferry *et al.*, 2009). Des études complémentaires sont donc nécessaires pour savoir si les ennemis naturels semblant être attirés et stimulés par les COVs peuvent effectivement diminuer les pressions d'herbivorie exercées par le ravageur sur la plante. Parallèlement, cette troisième étude a permis de mettre en évidence l'influence de deux COVs sur le comportement de *D. radicum* : le disulfure de diméthyle (DMDS) diminue l'infestation de la mouche du chou tandis que le (Z)-3-acétate d'hexényl (HA) l'augmente. Ces travaux sont les premiers à reporter un effet positif de HA sur la ponte de la mouche du chou et permettent de confirmer le rôle négatif du DMDS préalablement mis en évidence par Ferry *et al.*, 2007. Bien que ce troisième article constitue une première étape indispensable pour démontrer en conditions naturelles le rôle des COVs dans les interactions biologiques du réseau trophique étudié, nos observations se sont restreintes à l'influence des odeurs synthétiques sur l'infestation des plantes avoisinant directement les diffuseurs.

**Dans le quatrième article** nous avons intégré une analyse spatiale de l'influence des COVs sur l'infestation des plantes par *D. radicum*. Dans un contexte appliqué, les résultats que nous avons obtenus permettent de mieux comprendre comment déployer des diffuseurs de COVs de synthèse de façon à optimiser les modifications comportementales de l'herbivore et de ses ennemis naturels. Nous avons proposé deux hypothèses pour expliquer les effets des

COVs testés sur le nombre d'œufs pondus aux différentes distances étudiées. La première repose sur l'influence des COVs dans la réponse fonctionnelle de l'herbivore (*i.e.* l'oviposition). Des réponses de ce type sont le plus souvent dépendantes de la concentration du stimulus et devraient donc varier avec la distance au diffuseur (Schoonhoven *et al.*, 2005). Une deuxième hypothèse propose, quant à elle, une redistribution des effectifs. Alors que la densité d'un herbivore est constante à l'échelle de la parcelle, des zones devenues attractives grâce à un stimulus olfactif détecté dans une concentration adéquate devraient concentrer localement l'insecte au dépend des zones adjacentes (Braasch & Kaplan, 2012). Les différences de nombre d'œufs pondus aux différentes distances étudiées reposeraient alors sur ces dynamiques d'effectifs. En plus de l'oviposition, nous avons également estimé le nombre de pupes retrouvées par plant en fin de saison. Ce relevé permet d'avoir accès à l'infestation effective et reflète les pressions d'herbivorie réellement exercées sur la plante. Les différences d'infestation peuvent résulter de deux mécanismes : un contrôle « bottom-up » basé sur l'induction des défenses de la plante par les COVs de synthèse (Engelberth *et al.*, 2004) et un contrôle « top-down » reposant sur le recrutement des ennemis naturels par les stimuli olfactifs diffusés (James, 2005). De manière générale, ce quatrième article confirme le potentiel de trois composés, le DMDS, le HA et le MeSA dans la mise en place d'une stratégie « Push-Pull ». Alors que le DMDS réduit la ponte de *D. radicum* (« Push »), l'HA augmente l'infestation des plants par la mouche du chou (« Pull »). Le MeSA permet quant à lui de limiter les pressions d'herbivorie exercées sur la plante via des effets « bottom-up » ou « top-down ». Cette étude souligne néanmoins la complexité des effets de COVs de synthèse sur les interactions plantes - herbivores - ennemis naturels et la nécessité de conduire des études complémentaires (au laboratoire et sur le terrain, *cf.* perspectives) afin de déterminer les modes d'actions de certains COVs.

La mise en place de ceintures végétales autour de cultures d'intérêt constitue un second levier comportemental dont nous avons voulu tester l'efficacité dans **le cinquième article**. Trois types de ceintures ont été déployés autour de parcelles de brocoli. Nous avons retenu deux plantes stimulantes ou attractives vis-à-vis de *D. radicum* (Rousse *et al.*, 2003 ; Kergunteuil *et al.*, in prep.). Nos résultats montrent que la mise en place de ceintures de chou chinois (*B. rapa pekinensis*) permet de réduire l'infestation de la mouche du chou sur les plants de brocoli situés au centre et confirme le rôle protecteur d'une ceinture attractive. En revanche, le déploiement d'une ceinture de navet (*B. rapa*) ne permet pas de réduire l'infestation du brocoli. Cette ceinture a subi l'attaque de nombreuses autres espèces de

phytophages, ce qui a pu réduire son efficacité et souligne l'intérêt de considérer les interactions à l'échelle de la communauté et non des seuls insectes ciblés. Enfin, nous avons également voulu tester le potentiel d'une ceinture dissuasive avec la mise en place d'une ceinture de moutarde blanche, *S. alba* (cf. article n°1). Cette étude montre qu'un tel type de ceinture s'avère inefficace. Les plants de brocoli sont autant infestés avec ou sans ceinture de moutarde blanche. Nos résultats suggèrent qu'une ceinture de moutarde blanche ne s'apparente pas à une barrière physique et que la mouche du chou accède aux plants de brocoli malgré la présence de plantes dissuasives tout autour. D'autres modes de déploiement de la moutarde blanche sont probablement à envisager (e.g. en interculture).

Prise dans son ensemble, cette thèse a permis de montrer le potentiel des COVs dans la réorganisation des interactions plantes - insectes d'un agrosystème particulier. Les odeurs de plantes agissent de différentes façons à chacun des niveaux d'un réseau tri-trophique et constituent un levier comportemental prometteur pour protéger les cultures contre les insectes ravageurs. Dans le cadre du développement d'une stratégie de lutte basée sur la modification comportementale d'un insecte herbivore à l'aide de COVs de synthèse, nous avons proposé une démarche générale combinant quatre grandes étapes : (i) l'observation du comportement de l'insecte en conditions naturelles, (ii) la recherche au laboratoire de stimuli impliqués dans les prises de décisions comportementales, (iii) l'identification de substances sémiochimiques et (iv) leur utilisation au sein de parcelles expérimentales. Cette démarche devra par la suite s'accompagner d'une cinquième étape centrée sur une approche agronomique. En effet, l'efficacité d'une méthode de lutte contre un ravageur ne peut pas être établie tant que l'influence des leviers comportementaux sur la qualité des produits commercialisables n'a pas été estimée.

Au cours des vingt dernières années, les acquis de l'écologie chimique ont permis d'améliorer nos connaissances sur les interactions plantes – insectes et d'identifier de nombreux COVs susceptibles de modifier le comportement de différents insectes ravageurs; une part importante de ces connaissances obtenues au laboratoire peut d'ores et déjà être utilisée au sein de parcelles expérimentales. Cette thèse a également exploré le potentiel d'un second levier comportemental : la mise en place de ceintures végétales attractives en vue du développement d'une stratégie de lutte de type « Push-Pull ». Ce travail a mis en évidence l'intérêt de deux leviers comportementaux pour lutter contre la mouche du chou *D. radicum*. Cependant, nous avons pu nous rendre compte à travers les différentes expérimentations

menées dans cette thèse que plusieurs questions liées à l'utilisation de ces leviers restent à résoudre. Nous avons identifié dans la partie suivante cinq perspectives de recherche qu'il nous semble intéressant de développer dans un avenir proche : (i) la combinaison de ceintures végétales et de diffuseurs d'odeurs (ii) l'influence des conditions climatiques (iii) l'utilisation de mélange de COVs (iv) le mode d'action des COVs sur les insectes (v) la prise en compte du cortège d'herbivore présents dans l'environnement. La mise en place de ces perspectives pourrait permettre d'améliorer l'efficacité des leviers comportementaux utilisés dans cette thèse pour protéger les cultures de brassicacées contre *D. radicum*. Autour de cet objectif principal, ces cinq perspectives nous semblent également intéressantes pour affiner nos connaissances à propos des interactions plantes-insectes au sein des agrosystèmes.

## 2. Perspectives de recherche

### **Combiner des ceintures végétales avec des diffuseurs de COVs**

La combinaison de ceintures végétales et la diffusion de COVs synthétiques au sein de la même parcelle constitue la dernière étape vers la mise en place du « Push-Pull » assisté dont nous avons souhaité poser les bases au cours de cette thèse. L'efficacité de ces deux leviers comportementaux pris indépendamment l'un de l'autre pourrait être améliorée dans une méthode de lutte permettant de les combiner. Au-delà d'effets additifs, les méthodes « Push-Pull » reposent le plus souvent sur des effets synergiques entre plusieurs leviers (Cook *et al.*, 2007). Tandis que des COVs répulsifs placés dans une culture d'intérêt permettraient de limiter localement la présence de l'herbivore, des ceintures végétales assistées par des COVs attractifs pourraient faciliter la relocalisation du ravageur vers des zones périphériques. De plus, une sélection de plante piège appropriée peut permettre d'attirer le ravageur et simultanément favoriser son contrôle en limitant le développement de l'herbivore (contrôle « bottom-up ») ou en facilitant l'action des ennemis naturels (contrôle « top-down »). La mise en place d'une stratégie associant plusieurs leviers comportementaux au sein d'une parcelle réorganise en profondeur les interactions entre les herbivores, leurs ennemis naturels et leurs habitats. De telles modifications comportementales sont le plus souvent spécifiques et restent parfois difficiles à prédire (Khan *et al.*, 2008). Le scénario prometteur qu'il est possible d'envisager à travers le développement d'une stratégie « Push-Pull » destinée à lutter contre *D. radicum* nécessite d'être vérifié par des expérimentations en plein champs combinant des

ceintures pièges de chou chinois (*B. rapa pekinensis*) avec des diffuseurs de disulfure de diméthyle, de (Z)-3-acétate d'hexényl et de salicylate de méthyle. Les analyses chromatographiques que nous avons menées au cours de cette thèse permettent d'augmenter le registre de COVs synthétiques pouvant être utilisés au sein de ces parcelles expérimentales.

### **Influence des conditions climatiques**

Les travaux menés au cours de cette thèse ont permis de commencer à travailler sur les modalités de déploiement de COVs synthétiques à l'intérieur des parcelles. Nous avons mis en évidence (*cf.* article 4) l'importance de certains effets spatiaux sur l'infestation des plantes. Une prochaine étape intégrant des facteurs environnementaux apparaît nécessaire pour compléter ces premiers résultats. En effet, la diffusion d'odeurs synthétiques en conditions naturelles est fortement liée aux conditions climatiques comme la température, l'humidité ou le vent. L'utilisation de stations météorologiques dans les prochaines expérimentations de terrain apporterait plusieurs éléments de discussion intéressants dans un contexte appliqué. Au sein de parcelles comprenant des diffuseurs placés au centre, l'organisation spatiale des relevés pourrait ainsi être couplée avec l'enregistrement de la direction et de l'intensité des vents dominants. Plusieurs études ont souligné l'importance que peut avoir la direction du vent dans l'attraction des ennemis naturels à partir de stimuli olfactifs (*e.g.* Bernasconi-Ockroy *et al.*, 2001). Un protocole de ce type permettrait d'affiner nos conclusions sur les modalités de déploiement des diffuseurs à l'intérieur des parcelles et permettrait de mieux interpréter les spectres d'actions des différents COVs testés, que ce soit sur la modification comportementale de l'herbivore ou sur celle de ses ennemis naturels. Alors que l'importance du vent dans la réponse d'un insecte à un stimulus olfactif est reconnu depuis longtemps (*e.g.* Visser, 1988), des études plus récentes ont permis d'actualiser les connaissances sur l'influence de ce facteur environnemental dans la recherche de pistes olfactives par l'insecte (*e.g.* Cardé & Willis, 2008). Au-delà d'un aspect appliqué, un protocole expérimental intégrant les relevés de vent permettrait de mieux comprendre l'utilisation de signaux olfactifs par *D. radicum*.

### **Utilisation d'un mélange de COVs**

La plupart des études s'étant intéressées au potentiel des COVs de synthèse dans la protection des cultures se sont concentrées pour des raisons pratiques sur la diffusion de composés isolés. Cependant, les quelques auteurs ayant étudié la possibilité de diffuser des mélanges de

COVs suggèrent que de tels assemblages modifient plus efficacement le comportement des insectes. En effet, Tóth *et al.*, 2009 ont par exemple remarqué que l'ajout de salicylate de méthyle permet d'augmenter l'attractivité d'un mélange de deux COVs vis-à-vis de *Chrysoperla carnea* alors que cette chrysope ne répond pas au salicylate de méthyle lorsqu'il est isolé. Une approche intéressante dans l'assemblage de COVs attractifs a été proposée par del Soccoro *et al.*, 2010 qui ont étudié la possibilité de modifier le comportement d'un ravageur à partir d'un mélange de composés volatils émis par des plantes hôtes et des plantes non-hôtes utilisées par l'insecte pour se nourrir. La combinaison de COVs pour obtenir un « super-mélange » tentant de recréer des odeurs émises par des plantes hôtes, présente plusieurs avantages. La réponse d'un insecte à un stimulus olfactif dépend du contexte environnemental : des phénomènes d'apprentissage permettent à un insecte (qu'il soit phytophage ou entomophage) de répondre préférentiellement aux signaux olfactifs émis par un hôte abondant dans l'environnement plutôt qu'aux signaux olfactifs émis par un hôte peu présent à l'échelle locale (Webster *et al.*, 2012 ;Wei *et al.*, 2013). Un « super – mélange » pourrait s'avérer moins sensible à ces comportements d'apprentissage et permettrait d'être efficace dans des environnements contrastés. De plus, un mélange de COVs limitant les phénomènes d'apprentissage serait également utile dans un contexte de durabilité des stratégies basées sur la modification comportementale des insectes. Toutefois, il est important de souligner que la détermination des proportions relatives des COVs à inclure au sein d'un mélange reste souvent problématique (Kaplan, 2012).

### **Mode d'action des COVs**

Des études complémentaires pourraient également être mises en place afin de mieux cerner le mode d'action des COVs sur les réponses comportementales des insectes. Nous avons vu en introduction que les composés volatils d'origine végétale constituent au sein des réseaux trophiques des signaux susceptibles de modifier directement le comportement des insectes ou d'induire des modifications physiologiques chez la plante et donc d'agir indirectement sur les insectes (*i.e.* via des effets en cascade au sein de la chaîne trophique). Ces deux modes d'action des COVs ont souvent été pris en considération par les auteurs ayant réussi à modifier le comportement d'ennemis naturels à l'aide d'odeurs synthétiques. Cependant, à notre connaissance, aucune étude n'a permis de distinguer l'importance relative de ces deux scénarios écologiques. Des observations comportementales (tunnel de vol ou olfactométrie)



pourraient permettre d'estimer l'importance relative des deux modes d'actions des COVs. Il serait par exemple intéressant de tester l'influence de différents flux d'air sur le comportement d'un herbivore et/ou de ses ennemis naturels : (i) une odeur de plante (ii) une odeur de plante préalablement induite par un COV de synthèse (iii) une odeur de plante non induite et d'un COV de synthèse (*i.e.* le diffuseur étant placé après la plante dans le sens du flux d'air) (iv) l'odeur d'une plante induite et d'un COV de synthèse (*i.e.* le diffuseur étant placé avant la plante dans le sens du flux d'air).

### **Prendre en compte le cortège d'herbivores**

Enfin, le développement d'une stratégie de lutte ciblée contre *Delia radicum* devrait également intégrer l'influence des leviers comportementaux utilisés dans cette thèse sur le cortège d'herbivores s'attaquant aux brassicacées. Dans un contexte multi-trophique, il faudra s'assurer que les moyens mis en place pour lutter contre la mouche du chou ne présentent pas d'effets antagonistes favorisant la colonisation des parcelles par d'autres ravageurs comme la teigne des crucifères (*Plutella xylostella*), certaines altises (*Phyllotreta* spp.) ou certains pucerons (*Brevicoryne brassicae* ou *Mysus persicae*) dont nous avons pu observer la présence au sein des parcelles expérimentales.

## **3. Conclusion générale**

Pour conclure, le travail apporté par cette thèse a permis de confirmer le potentiel des COVs dans la protection des cultures et a démontré l'intérêt d'insérer ces leviers comportementaux au sein d'une méthode de lutte prometteuse, le « Push-Pull ». Nos premiers résultats sont encourageants et permettent d'ouvrir de nouvelles pistes de recherches vers la mise en place d'une stratégie de lutte efficace contre *D. radicum*. L'étude de la réorganisation des réseaux trophiques au sein des stratégies « Push-Pull » apporte de nombreuses perspectives de recherches en écologie. Ce cadre conceptuel semble propice au rapprochement de l'écologie « académique » avec l'étude des écosystèmes anthropisés (INRA, 2012). En effet, l'approche développée au cours de cette thèse a permis d'intégrer dans nos discussions de nombreuses interactions entre les organismes vivants présents dans un agrosystème. Au fur et à mesure que nous progressons dans l'étude des mécanismes impliqués au sein des communications plante-herbivore-ennemis naturels, nous posons

également de nouvelles questions. De nombreuses recherches restent à mener pour améliorer notre connaissance sur l'ensemble des processus écologiques en jeu au sein des parcelles et parvenir à un mode de gestion durable et efficace des insectes ravageurs.

Dans un contexte de réorganisation des systèmes de production agricole, les stratégies « Push-Pull » constituent un outil supplémentaire dans le développement de solutions alternatives à la lutte chimique. Ce type de stratégie intègre les deux grands principes sur lesquels repose l'agroécologie : l'accroissement de la biodiversité et le renforcement des régulations biologiques (Wezel *et al.*, 2009 ; Schaller, 2013). En effet, la modification de l'habitat et la mise en place de ceintures végétales au sein des parcelles s'appuient sur le premier principe. L'utilisation de COVs s'inspire, quant à elle, des services écosystémiques impliqués dans le contrôle des populations d'insectes herbivores. L'engagement pris par les pouvoirs publics pour développer de nouveaux moyens de production agricole (Le Foll S., 2013) encourage les recherches à venir.



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**Le Président de Jury,**

## RESUME

Les composés organiques volatils (COVs) émis par les végétaux jouent un rôle crucial dans les interactions plante-insectes en contribuant à l'organisation des réseaux trophiques. De nombreuses études tendent à montrer que les COVs issus des plantes peuvent être exploités par l'Homme pour le développement de stratégies de protection des cultures contre les insectes ravageurs. Les COVs, émis naturellement par une plante ou diffusés artificiellement, peuvent notamment être intégrés dans une méthode de type « Push–Pull » visant à repousser l'herbivore de la culture d'intérêt (composante « Push ») pour l'attirer en périphérie de la parcelle (composante « Pull »). Au cours de cette thèse, l'objectif fut d'évaluer le rôle potentiel des COVs dans la mise en place d'une telle méthode pour protéger les cultures de Brassicacées contre l'un de ses principaux ravageurs, la mouche du chou *Delia radicum*. Plusieurs étapes ont été menées de front, au laboratoire et au sein de parcelles expérimentales, pour i) explorer le registre de stimuli olfactifs impliqués dans la structuration du système tri-trophique considéré, ii) étudier la mise en place de leviers comportementaux permettant d'alimenter les deux composantes de la stratégie « Push-Pull ». Nos expériences au laboratoire ont révélé que les odeurs émises par différentes Brassicacées influencent le choix d'un site de ponte chez la mouche du chou expliquant ainsi les différents taux d'infestation observés en condition naturelle (article 1). Des analyses chimiques en chromatographie en phase gazeuse ont par ailleurs permis de montrer que les plantes les plus attractives sont caractérisées par une émission abondante de certains sesquiterpènes (article 2). Ainsi l'utilisation d'une brassicacée attractive, le chou chinois, disposée en ceinture d'une parcelle de brocoli permet de réduire l'infestation du ravageur dans la parcelle cible (article 5). Nos résultats ont également permis de montrer que la diffusion de COVs de synthèse sur des parcelles expérimentales entraîne une modification des taux d'infestation (article 3) à plus ou moins longue distance des diffuseurs (article 4) et peut également avoir un effet bénéfique sur la présence des ennemis naturels de l'herbivore (article 3&4). Le disulfure de diméthyle (DMDS) diminue l'infestation tandis que le (Z)-3-acétate d'hexényl (HA) l'augmente. Le salicylate de méthyle (MeSA) permet quant à lui de limiter les pressions d'herbivorie exercées sur la plante via des effets « bottom-up » ou « top-down ». Ce travail de thèse montre donc que l'utilisation d'odeurs (naturelles ou artificielle) de plantes peut permettre de modifier la distribution d'un ravageur en plein champ. Cette étude aura ainsi permis d'ouvrir des perspectives intéressantes pour la mise en place d'une stratégie de type « Push-Pull assisté » où des variétés plus ou moins attractives pourraient être combinées à des COVs de synthèse pour repousser le ravageur de la parcelle d'intérêt afin de l'attirer en périphérie de celle-ci.

**Mots clés :** composé organique volatil, interaction plante-insecte, réseau trophique, « Push-Pull », Brassicacées, *Delia radicum*.