Décrypter les mécanismes de reconnaissance et d'acceptation d'une plante hôte par un insecte phytophage spécialiste

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UNIVERSITÉ DE RENNES 1

THESE DE DOCTORAT DE

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Rapporteurs avant soutenance :

Frédéric Marion-Poll	Professeur - AgroParisTech
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Composition du Jury :

Président :	Philippe Lucas	Directeur de recherche INRAE – iEES Versailles
Examinateurs :	Florence Nicolè	Maitre de conférences – Université Jean Monnet
	Nicole van Dam	Professeure – Université de Jena
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Partis bien trop vite ou en cours de route.
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Table des matières

Introduction générale

I. La spécialisation trophique chez les insectes phytophages

Les insectes sont le taxon animal le plus diversifié, avec actuellement plus d'un million d'espèces décrites et une estimation totale d'environ 5,5 millions (Stork, 2018). Près de la moitié d'entre eux sont phytophages (Strong *et al.*, 1984), c'est-à-dire qu'ils exploitent des plantes pour compléter leur cycle de développement. Chaque insecte phytophage a sa propre gamme d'hôtes mais une très grande majorité d'entre eux (plus de 90 %) s'est spécialisée sur une ou quelques familles de plantes (Bernays et Graham, 1988). On dit de ces espèces qu'elles sont spécialistes, en opposition aux généralistes qui exploitent un large nombre de familles végétales. Les insectes phytophages sont une source importante de stress pour les plantes, en raison de la diminution de fitness causée par exemple par la perte d'organes reproducteurs, ou plus directement par la mort. En réponse à la pression de phytophagie qu'elles subissent, les plantes ont développé différents mécanismes de défense, complexes et multiples, tels que la production de barrières physiques et surtout chimiques. En effet, elles peuvent produire des composés qui peuvent être toxiques et ainsi affecter la survie ou la fécondité des insectes (Vancanneyt *et al.*, 2001, Hartmann, 2007), qui peuvent réduire leur digestibilité ou encore qui peuvent agir comme répulsifs (De Moraes *et al.*, 2001 ; Kessler et Baldwin, 2001 ; Mello et Silva-Filho, 2002 ; War *et al.*, 2012) sur les herbivores qui les consomment. Certains composés de défense peuvent également agir de façon indirecte en favorisant l'attraction des ennemis naturels de l'herbivore (Dicke, 1999 ; McCormick *et al.*, 2012). En parallèle, les insectes ont développé des stratégies pour surmonter ces barrières végétales. En particulier, les insectes spécialistes ont souvent acquis des adaptations comportementales, métaboliques et génétiques qui leur permettent de faire face aux systèmes de défense des plantes, et ainsi d'avoir de meilleures performances lorsqu'ils se nourrissent de ces plantes en comparaison d'espèces généralistes ou spécialisées sur d'autres plantes. Par exemple, certains spécialistes sont capables d'éviter la formation des composés toxiques végétaux (Pentzold *et al.*, 2014), de les détoxifier (Heidel-Fisher et Vogel, 2015) ou encore de les séquestrer sans les activer, parfois à leur propre avantage face à leurs ennemis naturels (Duffey, 1980 ; Müller *et al.*, 2001). Cette spécialisation a également conduit au développement de capacités d'exploitation de signaux physiques et chimiques spécifiques des plantes de leur gamme d'hôtes, permettant ainsi aux phytophages de les reconnaitre dans leur environnement. Parmi les signaux physiques, la taille de la plante, sa forme ou encore sa couleur peuvent offrir quelques indices aux insectes sur son identité et sa localisation, majoritairement à distance (Renwick et Chew, 1994 ; Calatayud *et al.*, 2008 ; Whitney *et al.*, 2009). Cependant, ces indices physiques sont peu spécifiques et peuvent varier au sein d'une même espèce et même pour un même individu au cours du temps (Eisikowitch et Rotem 1987, de Melo *et al.,* 2018). Il est donc généralement admis que ces signaux, seuls, ne permettent pas aux insectes de reconnaitre une plante hôte (Prokopy et Owens, 1983). Les signaux prépondérants dans la reconnaissance et la sélection de l'hôte sont ainsi les composés chimiques

produits par les plantes, et particulièrement les métabolites spécialisés (anciennement « secondaires »), dont la répartition taxinomique réduite est utilisée comme marqueur d'appartenance à la famille ou l'espèce hôte (Bernays et Chapman, 1994 ; Schoonhoven *et al.,* 2005).

II. La reconnaissance et l'acceptation de la plante en milieu aérien

1. Les différentes étapes de la sélection chez les adultes

Les insectes phytophages exploitent les plantes à la fois pour se nourrir et pour se reproduire. Suivant leur stade de développement ou leur état physiologique, ils seront à la recherche soit d'une source de nourriture, soit d'un site d'oviposition. La nature des signaux végétaux utilisés dans le processus de sélection de l'hôte dépendra alors de la ressource recherchée. Dans ce processus, deux grandes phases comportementales consécutives peuvent être distinguées : la recherche à distance et l'évaluation au contact. La première phase peut se terminer par la localisation de la plante et le rapprochement de l'insecte vers celle-ci, la seconde par son acceptation ou son rejet.

A. La recherche à distance

Avant de pouvoir entrer en contact avec une plante hôte, les insectes phytophages doivent tout d'abord la rechercher dans leur environnement et s'en approcher. Toutes les plantes libèrent dans leur environnement un mélange de composés organiques volatils (COV), des métabolites spécialisés intervenant de façon importante dans cette phase de l'interaction (Visser, 1986).

L'ensemble des COV émis par une plante forme un 'bouquet d'odeurs', qui peut parfois contenir jusqu'à une centaine de composés différents (Dudareva *et al.*, 2006) bien que souvent le mélange soit dominé par un ou quelques composés majoritaires (Van den Boom *et al.*, 2004 ; Blaakmeer *et al.*, 1994 ; Van Poecke *et al.*, 2001). Ces COV sont divisés en quatre grandes classes selon leur voie de biosynthèse : les terpènes, les composés aromatiques ou shikimiques, les composés verts ou dérivés d'acides gras, et les composés soufrés. Chaque mélange de COV produit par une plante individuelle constitue un signal olfactif unique dont les qualités perceptives sont en partie fonction du nombre de composés, de leur concentration respective et de leurs proportions relatives dans le mélange (Wright et Smith 2004a, b). On s'attend à ce que la différence dans les signaux olfactifs soit généralement plus grande entre différentes espèces qu'au sein d'une même espèce, et donc à ce que les insectes discriminent plus facilement deux espèces que deux populations.

Les environnements naturels étant très riches en information, le système olfactif des insectes est adapté pour en extraire les informations pertinentes. Ainsi pour une espèce et un stade physiologique donnés, seule une minorité des composés des bouquets d'odeurs est détectée par les récepteurs olfactifs **[Encart 1]** et une partie seulement des composés perçus déclenchent ensuite une réponse comportementale. L'attraction des adultes phytophages en milieu aérien est bien décrite dans la littérature depuis les travaux de Visser et Avé (1978). Elle peut être médiée par un ou quelques composés taxinomiquement caractéristiques, comme cela a été observé avec des COV spécifiques d'Asteraceae (Krasnoff et Dussourd 1989), d'Apiaceae (Guerin *et al.*, 1983), d'Alliaceae (Judd et Borden, 1989), de Brassicaceae (Nottingham *et al.*, 1991 ; Blight *et al.*, 1995 ; Baoyu *et al.*, 2001), de Cucurbitaceae (Bjostad et Hibbard, 1992) ou encore de Rosaceae (Knight et Light, 2001). Il a également été démontré que des insectes appartenant à plusieurs ordres possédaient des récepteurs olfactifs capables de détecter des COV ubiquistes tels que des dérivés d'acides gras, des phénylpropanoïdes ou des isoprénoïdes (Hansson *et al.*, 1999 ; Bruce *et al.*, 2005). Même s'ils sont émis par des plantes très différentes, ces composés ubiquistes peuvent participer à la spécificité du signal pour les insectes spécialistes car ils sont rarement émis dans les mêmes proportions par toutes ces plantes. Ce sont alors les proportions relatives de ces composés dans le bouquet d'odeurs qui deviennent importantes et permettent la reconnaissance (Webster *et al.*, 2008a, 2008b ; Bruce et Pickett, 2011) (Fig. 2). Par ailleurs, des études suggèrent que certains COV produits par la plante sont essentiels à sa reconnaissance tandis que d'autres peuvent être remplacés sans changer la nature attractive du bouquet (Nojima *et al.*, 2003 ; Tasin *et al.*, 2007 ; Cha *et al.*, 2008) (Fig. 2). Ces composés sont alors dits 'redondants'. Cette redondance est illustrée par exemple chez les femelles de l'eudémis de la vigne (*Lobesia botrana*). Tasin *et al.* (2007) ont présenté aux femelles un mélange de 10 COV émis par le raisin de son hôte *Vitis vinifera* précédemment identifié comme attractif (Tasin *et al.*, 2006). Au cours de leur étude, ils ont retiré des COV au bouquet initial et ont constaté que l'attraction exercée par un mélange de trois composés, (E)-caryophyllène, (E)-β-farnésène et (E)-4,8-diméthyl 1,3,7-nonatriène (DMNT), n'était pas significativement différente de l'attraction exercée par le mélange initial de 10 COV. Si l'un de ces trois composés était retiré du mélange à trois COV, l'attraction diminuait considérablement. De plus, il était possible de remplacer chacun de ces trois composés par d'autres composés du raisin pour obtenir un mélange presque aussi attractif que le mélange initial. Cet exemple illustre à la fois le fait qu'un ou quelques composés particuliers peuvent être essentiels pour la reconnaissance tandis que d'autres, moins importants peuvent être absents, et que des composés redondants peuvent se substituer les uns aux autres.

Encart 1 : Les récepteurs sensoriels des insectes

Les insectes phytophages détectent les composés chimiques produits et émis par les plantes avec leurs sensilles chimioréceptrices. Ces organes sont constitués de cellules accessoires, de neurones récepteurs et d'une structure cuticulaire abritant les dendrites de ces neurones (Fig. 1).

Les **chimiorécepteurs olfactifs** (ou sensilles olfactives) sont principalement rencontrés sur les antennes et parfois sur les pièces buccales (comme chez les Orthoptères (Bland, 1991)) et même en petit nombre sur l'ovipositeur de certaines femelles (Yadav et Borges, 2017). Ces sensilles possèdent de nombreux pores disséminés sur leur cuticule (Fig. 1A) et permettent aux insectes de détecter les molécules volatiles.

Les **chimiorécepteurs gustatifs** (ou sensilles gustatives) sont majoritairement situés sur les pièces buccales, les extrémités des pattes et l'ovipositeur des femelles, mais aussi parfois sur les ailes, les antennes et tout le reste du corps (Chapman, 2003). Ces récepteurs sont sensibles aux composés en solution dans un liquide ou adsorbés à la surface de substrats plus secs comme les feuilles (Pietra *et al.*, 1980 ; Shields *et al.*, 1994). Ils permettent de détecter les composés de surface ou internes, et au contraire des récepteurs olfactifs, ils ne possèdent qu'un pore apical (Altner et Prillinger, 1980) (Fig 1B).

Lorsque les molécules pénètrent dans les sensilles, elles diffusent dans l'hémolymphe (soit grâce à leurs propriétés hydrophiles, soit à l'aide de protéines de transport) et atteignent les terminaisons nerveuses. Si ces molécules sont reconnues par les récepteurs membranaires des dendrites des neurones, une dépolarisation membranaire de ces dendrites aura lieu, induisant une transduction du signal chimique en un signal électrique acheminé par les neurones vers les centres nerveux de l'insecte – où le signal sera intégré. Chaque neurone récepteur exprime un seul type de récepteur membranaire, capable de se lier avec une gamme plus ou moins grande de molécules. Cependant, même si un neurone permet de détecter plusieurs types de molécules, la gamme à laquelle il répond reste limitée. Cela permet ainsi à l'insecte de ne pas intégrer tous les signaux chimiques de son environnement mais simplement ceux qui peuvent être perçus par son système sensoriel. Ce n'est qu'une fois le signal intégré au niveau du système nerveux central que celui-ci déclenchera, ou non, un comportement (Sachse et Krieger, 2011 ; Szyszka et Galizia, 2015 ; Renou et Anton, 2020).

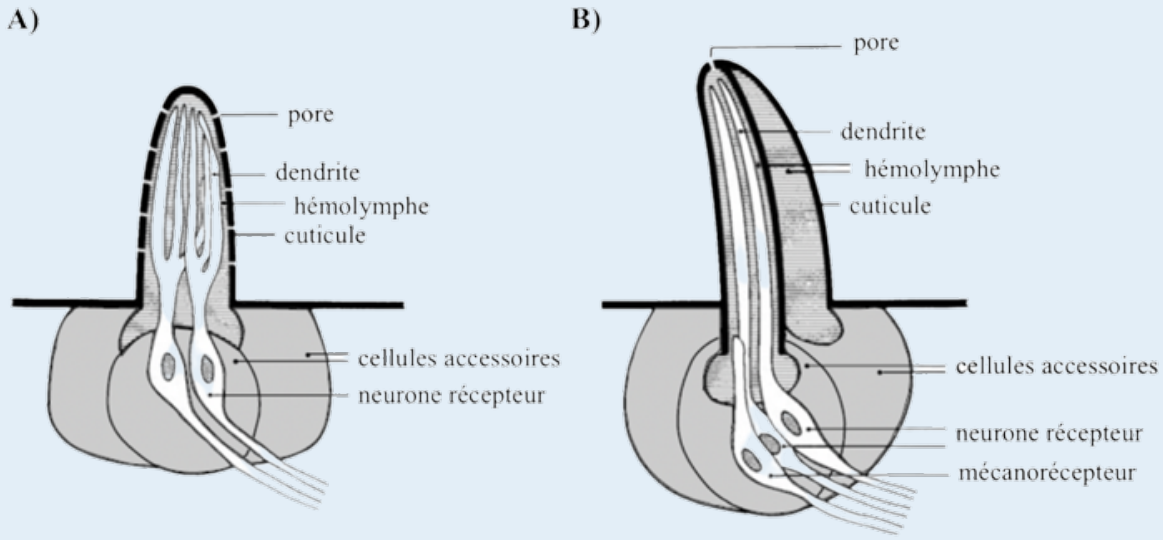


Figure 1 : Schéma de sections longitudinales (A) d'une sensille olfactive et (B) d'une sensille gustative d'insecte (modifié d'après Schoonhoven *et al.* (2005))

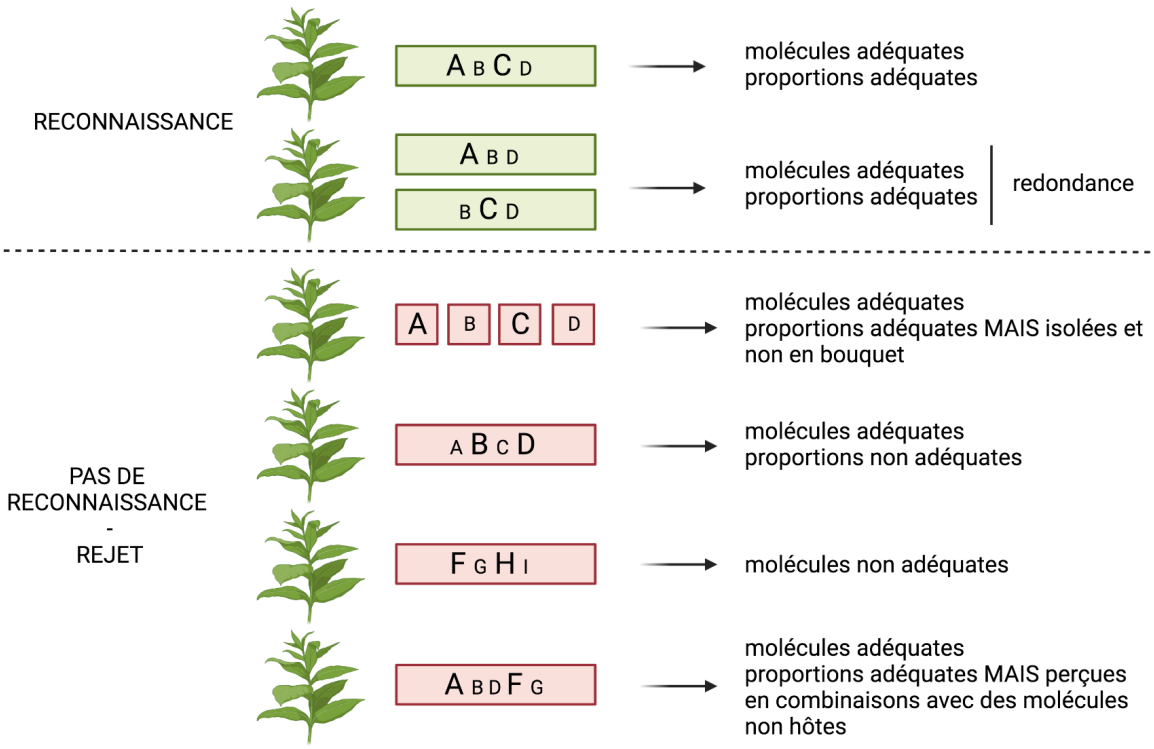


Figure 2. Schématisation de la reconnaissance d'un signal olfactif caractéristique de l'hôte par un insecte phytophage à partir de composés organiques volatils (COV) ubiquistes (inspiré de Bruce et Pickett, 2011). Les différents COV détectés et leurs proportions relatives sont schématisés par des lettres et des tailles différentes qui représentent respectivement leur identité et abondance relative.

B. L'évaluation au contact

Une fois qu'un insecte est arrivé sur une plante, il doit l'évaluer et décider de l'accepter ou non. L'acceptation d'une plante comme hôte se traduit par une stimulation des comportements d'alimentation ou de ponte. Les composés de contact, qui agissent lors de cette phase, sont détectés par des récepteurs sensoriels gustatifs **[Encart 1]** et peuvent avoir des effets positifs (c'est-à-dire stimulants) ou négatifs (c'est-à-dire dissuasifs) sur le comportement des insectes. C'est l'équilibre entre la détection et l'intégration de ces stimuli positifs et négatifs qui déterminera l'acceptation finale ou le rejet de la plante (Machado *et al.*, 2021). Dans le cas de l'alimentation par exemple, les neurones associés aux récepteurs gustatifs seraient associés à des cellules phagostimulatrices et à des cellules dissuasives et c'est l'activité de ces cellules, en réponse à des stimuli qu'elles sont capables de reconnaître, qui stimulerait ou inhiberait l'alimentation (Chapman, 2003).

L'implication des composés de surface

Avant de se nourrir ou de pondre, de nombreux insectes herbivores présentent des séquences comportementales stéréotypées d'échantillonnage de la surface de la plante (Bernays et Simpson, 1982 ; Miller et Strickler, 1984). La première structure végétale que rencontrent les insectes lors de la phase de contact avec la plante est en effet la cuticule, particulièrement les cires épicuticulaires qui représentent la partie la plus superficielle de cette cuticule. Ces cires constituent l'enveloppe protectrice la plus externe de la plante et sont principalement composées d'alcanes à longue chaîne, d'esters, d'acides gras libres, d'alcools primaires et secondaires et d'aldéhydes (Müller et Riederer, 2005). Malgré la faible perméabilité de ces membranes lipidiques aux composés polaires, des métabolites internes polaires comme des sucres ou des acides aminés peuvent se retrouver, par diffusion, dans les cires épicuticulaires (Schönherr 2006 ; Zeisler-Diehl *et al.*, 2020). Ces composés sont généralement communs à de nombreuses plantes mais les cires peuvent également contenir des substances chimiques caractéristiques de la plante. En effet, des métabolites spécialisés non volatils peuvent se retrouver en surface de la plante par diffusion à travers la cuticule, ou bien être stockés dans les trichomes glandulaires (Bargel *et al.*, 2004). Tous ces composés peuvent fournir des informations sur le stade phénologique de la plante, son état physiologique ou ses interactions antérieures avec d'autres organismes (Chapman, 2003 ; Mamrutha *et al.*, 2017 ; Tomasi *et al.*, 2018). La qualité et la quantité des cires épicuticulaires pouvant varier fortement entre différentes espèces (Griffiths *et al.*, 2001), ces différences peuvent également donner des informations sur l'identité de la plante aux insectes, leur permettant ainsi de distinguer leur plante hôte de plantes non hôtes ou d'établir une hiérarchie de préférence entre différentes plantes hôtes. Par exemple, Hora et Roessingh (1999) ont montré que les femelles du grand hyponomeute du fusain (*Yponomeuta cagnagellus*), préfèrent fortement les extraits méthanoliques de surface de leur hôte à ceux de deux espèces de plantes non hôtes, suggérant des capacités de reconnaissance des signaux chimiques de surface associés à l'hôte chez cette espèce. D'autre part, des variations dans la composition des cires épiculaires sont également observées au sein d'une même espèce (Griffiths *et al.*, 2001 ; Hopkins *et al.*, 1997), pouvant ainsi permettre aux insectes de reconnaître différentes populations et donc de choisir l'hôte le plus approprié. Par exemple, une étude sur l'effet des extraits de surface foliaire de deux génotypes de blé (*Triticum aestivum*) sur l'oviposition de la mouche de Hesse (*Mayetiola destructor*) a montré que le contraste de ponte observé entre les deux génotypes était associé à des différences dans la composition chimique de leurs cires épicuticulaires (Cervantes *et al.*, 2002). Lorsqu'il est reconnu comme associé à un hôte, le signal perçu au contact de la plante entraîne l'acceptation de la plante qui peut se traduire par exemple par la stimulation de l'oviposition chez les femelles. Ainsi, de nombreux exemples de stimulation de l'oviposition en réponse à des extraits de surface foliaire de plantes hôtes ont été rapportés chez des insectes comme la piéride du chou *Pieris rapae* (Renwick et Radke, 1983), *Heliothis subflexa* (Mitchell et Heath, 1987), la teigne du poireau *Acrolepiopsis assectella* (Auger et

Thibout, 1983), la mouche du sorgho *Atherigona soccata* (Unnithan *et al.*, 1987) et l'oxyure de la tomate *Keiferia lycopersicella* (Burton et Schuster, 1981). De nombreuses études utilisent une approche de fractionnement bioguidé afin d'identifier plus rapidement la nature (ou polarité) des composés à l'origine de l'effet biologique observé **[Encart 2]**.

Les mécanismes de reconnaissance

De la même façon que l'attraction, l'acceptation d'une plante hôte peut être basée sur la présence d'un ou quelques composés spécifiques ou bien sur une combinaison de composés ubiquistes. Dans certaines études l'influence d'un seul type de composé spécifique est mise en évidence, comme dans le cas du papillon de la bougresse, *Junonia coenia*, qui répond à des glycosides iridoïdes, caractéristiques de la famille des Plantaginaceae (Pereyra et Bowers, 1988). Dans d'autres études, une combinaison de plusieurs composés semble être nécessaire pour stimuler la ponte. C'est le cas chez certaines espèces de Papilionidae qui exploitent un nombre limité de plantes parmi les Rutaceae, Apiaceae ou Lauraceae, pour lesquelles une combinaison spécifique de composés de leurs plantes hôtes est nécessaire pour déclencher la ponte (Honda, 1990 ; Murakami, 2003). Par exemple, chez les femelles du papillon *Papilio xuthus*, un mélange de six à 10 composés produits par les feuilles d'agrumes est nécessaire pour induire la ponte, aucun d'entre eux n'étant suffisant seul (Nishida *et al.*, 1987 ; Ohsugi, *et al.*, 1991). En plus de stimuler la ponte, la teneur en composés de surface des feuilles peut, chez certaines espèces, être corrélée avec les préférences de ponte. C'est le cas de la teneur en glycosides phénoliques chez la tenthrède *Nematus oligospilus* (Fernández *et al.*, 2019), des glucosinolates chez *Pieris napi macdunnoughii* (Rodman et Chew, 1980) ou des glycosides de flavonol chez le papillon monarque *Danaus plexippus* (Haribal et Renwick, 1998). Cependant, malgré de nombreuses études sur l'implication des composés de surface dans la stimulation de l'oviposition, beaucoup d'entre elles se focalisent soit sur une ou quelques espèces de plantes, soit sur quelques populations, mais peu portent sur les deux échelles à la fois (exemples dans Riederer et Müller, 2006). Or, puisque les femelles de nombreuses espèces de phytophages ont des préférences d'oviposition pour certaines espèces voire certaines populations d'une même espèce, c'est qu'un signal leur permet de discriminer un hôte d'un non hôte et aussi un hôte d'un autre hôte, et ainsi d'adapter leur comportement en fonction du type de signal perçu. Pour comprendre quel signal est impliqué dans l'acceptation elle-même, mais aussi quel signal est impliqué dans les contrastes d'acceptation, il est donc nécessaire d'étudier les deux échelles inter- et intraspécifiques de concert.

Encart 2 : L'approche de fractionnement bioguidé chez les insectes phytophages

L'approche de fractionnement bioguidé est utilisée pour isoler les composés d'un mélange qui sont biologiquement actifs sur un insecte d'intérêt (Araniti *et al.*, 2013 ; Fathallah *et al.*, 2019). Pour cela, un extrait total du matériel végétal (ex : feuilles, racines) est réalisé, puis son effet sur le comportement de l'insecte est testé à l'aide d'un dispositif expérimental sur/dans lequel l'extrait est déposé (Unnithan *et al.*, 1987 ; Shimoda et Kiuchi, 1998). Si l'insecte répond à l'extrait total, celui-ci sera alors fractionné – souvent en fonction de la polarité des molécules – et les différentes fractions seront testées de la même manière, seules ou en combinaison. L'objectif est ainsi d'isoler la ou les fractions causant l'effet biologique de l'extrait total (Renwick et Radke, 1983) (Fig. 3). Le fractionnement peut continuer en réduisant de plus en plus le mélange initial de composés, jusqu'à déterminer une fraction minimale contenant les composés biologiquement actifs et ensuite pouvoir réaliser des analyses métabolomiques et identifier des composés candidats à tester par la suite. Cette procédure de fractionnement bioguidé est indispensable lorsque l'on souhaite ne pas avoir d'*a priori* sur la nature des molécules qui induisent le comportement observé.

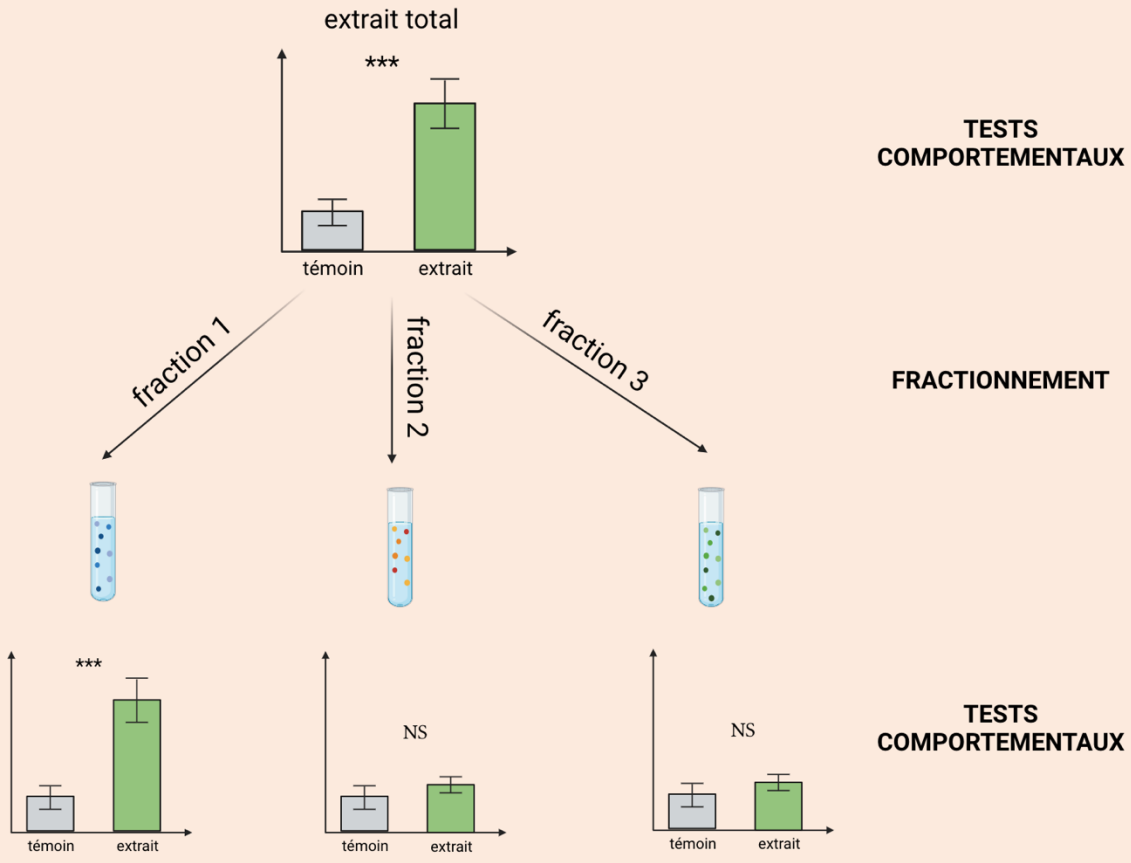


Figure 3 : Représentation schématique de la méthode de fractionnement bioguidé (*** = réponse biologique significative de la part de l'insecte, NS = pas de réponse significative).

Le rôle des COV au contact

Bien que les COV soient surtout connus pour jouer un rôle clé dans l'attraction des insectes à distance, ils peuvent dans certains cas agir en synergie avec les composés de surface et ainsi renforcer le message perçu par les insectes, induisant une reconnaissance plus fiable car basée sur une diversité de signaux. Cette synergie a pu être mise en évidence à la fois chez des phytophages spécialistes et généralistes. Elle a pu être observée par exemple chez *Cassida stigmatica*, une espèce de chrysomèle monophage (Müller et Hilker, 2001), chez *Nematus oligospilus*, une tenthrède spécialiste des Salicaceae (Braccini *et al.*, 2015) ou encore chez le papillon de la vigne *Lobesia botrana*, un phytophage généraliste dont la vigne est l'un des hôtes (Tasin *et al.*, 2011). Dans d'autres cas, il a pu être montré que la présence seule de COV suffisait à stimuler l'oviposition (Damodaram *et al.*, 2014 ; Dwerk *et al*, 2013 ; Feng *et al.*, 2017) ou à la réduire (Koschier et Sedy, 2001 ; Koul *et al.*, 2013). Cependant, les études sur l'effet des COV au contact restent encore minoritaires par rapport celles sur leur effet à distance. De plus, les études en question se focalisent majoritairement sur la finalité du comportement (*i.e.* la stimulation de l'alimentation ou de l'oviposition), très peu sur son initiation qui est pourtant indispensable. Les COV peuvent en effet être à l'origine de l'initiation de certains comportements lors du contact entre l'insecte et la plante. Par exemple, Müller et Hilker (2001) ont étudié l'effet des COV de la tanaisie commune (*Tanacetum vulgare*) sur le comportement de la casside *Cassida stigmatica*, chez qui les adultes grimpent sur les pétioles des jeunes feuilles à la recherche de sites d'alimentation et de ponte. Dans cette étude, les auteurs ont réalisé des observations comportementales au cours desquelles ils confrontaient des pétioles artificiels, chimiquement neutres, à des pétioles de feuilles de tanaisie commune enroulés dans du papier filtre afin que les insectes ne soient pas au contact direct des signaux de surface (et n'aient à leur disposition que des indices olfactifs). Il a été observé une marche plus longue sur les vrais pétioles enroulés dans du papier filtre que sur les pétioles artificiels, prouvant l'intérêt de l'insecte pour la plante à partir de la seule détection des COV et sans contact direct celle-ci. Cette étude, et d'autres, suggèrent que l'effet des COV dans la sélection de l'hôte ne se limite pas seulement ni à la recherche à distance, ni à la synergie avec les composés de contact. Dissocier, de manière plus systématique, les effets des COV à distance et au contact de la plante permettrait ainsi de mieux distinguer les signaux précis impliqués à chaque étape comportementale de la sélection de l'hôte.

2. L'influence de l'état physiologique des adultes sur leur réponse aux signaux volatils

La réponse des insectes aux signaux de leur environnement dépend d'un certain nombre de facteurs exogènes (par exemple le vent ou la pression atmosphérique), mais aussi de facteurs endogènes qui font que dans un même environnement, la réponse comportementale peut différer selon l'état physiologique

de l'individu. Cette plasticité aide les insectes à prendre des décisions optimales en fonction de leur état physiologique, par exemple pour les réponses aux phéromones sexuelles ou aux signaux émis par les sites de ponte (pour optimiser la reproduction), ou les réponses aux signaux émis par les plantes utilisées comme source alimentaire.

Parmi les facteurs endogènes, l'âge ainsi que la maturité sexuelle sont connus pour avoir un effet sur la détection des COV (Gadenne *et al.*, 2016). Chez beaucoup d'espèces d'insectes, une période spécifique après l'émergence est nécessaire pour entrer en phase de reproduction. La maturation sexuelle des femelles s'accompagne alors de modifications physiologiques pour produire des œufs. Des études ont pu montrer que les capacités de réponse aux différents stimuli émis par les plantes sont directement liées à l'âge et donc à la maturité sexuelle. Par exemple, les femelles non accouplées de la noctuelle méditerranéenne *Spodoptera littoralis* sont fortement attirées par les fleurs de lilas (*Syringa vulgaris*), tandis qu'après l'accouplement l'attraction pour l'odeur florale est abolie et les femelles se dirigent préférentiellement vers l'odeur des feuilles vertes de la plante où se développent leurs larves, le coton (*Gossypium hirsutum*) (Saveer *et al.*, 2012). De même, les femelles non accouplées de la mouche des fruits du Queensland, *Bactrocera tryoni*, sont plus réceptives à des odeurs de phéromones mâles alors que les femelles accouplées sont plus réceptives à des odeurs de fruits. Ceci suggère un changement de préférence olfactive après l'accouplement, qui se traduirait par un passage du comportement de recherche de mâles à celui de la recherche de fruits pour y déposer leurs œufs (Devescovi *et al.*, 2021).

Les réponses aux signaux volatils peuvent également dépendre du sexe de l'insecte. Par exemple chez le carpocapse *Cydia pomonella*, lorsque des doses comprises entre 60 et 12 000 ng d'alpha-farnésène sont testées, les femelles sont attirées par de faibles doses et repoussées par de fortes doses, contrairement aux mâles pour lesquels ni attraction ni répulsion n'ont été observés (Hern et Dorn, 1999). Une attractivité plus forte des COV de la plante hôte a également été observée chez les femelles accouplées de différentes espèces de papillons de nuit par rapport aux mâles, par exemple *Trichoplusia ni* (Landolt, 1989), *Pectinophora gossypiella* (Wiesenborn et Baker, 1990), *Heliothis subflexa* (Tingle *et al.*, 1989) et *Heliothis virescens* (Tingle et Mitchell, 1992). De plus, chez les espèces phytophages où les mâles adultes ne se nourrissent pas, les COV de la plante hôte seuls sont rarement attractifs, comme par exemple chez l'eudémis de la vigne *Lobesia botrana* (Masante-Roca *et al.*, 2007). Le plus souvent, les odeurs de la plante hôte agissent sur les mâles en synergie avec les phéromones sexuelles, comme un indice supplémentaire pour localiser les femelles (Reddy et Guerrero, 2004).

D'autres facteurs qui ne seront pas développés ici comme la charge en œufs (Odendaal, 1989 ; Odendaal et Rausher, 1990), l'état nutritionnel (Barton Browne, 1993 ; Hern et Dorn, 1999 ; Dkhili *et al.*, 2019) ou l'expérience (Anderson et Anton, 2014) peuvent également moduler la réponse des insectes aux signaux volatils.

3. Les signaux utilisés par les larves pour choisir une plante

Pour survivre, les larves doivent évaluer correctement la qualité de leur hôte et ajuster leur comportement en fonction des composés chimiques qu'elles détectent. Bien qu'elles ne disposent que d'un nombre limité de chimiorécepteurs en comparaison des adultes (Schoonhoven, 1987), les larves en sont bel et bien équipées. En particulier, elles possèdent des sensilles olfactives qui leur permettent de détecter des composés volatils (Dethier et Schoonhoven 1969). L'effet des COV sur le comportement des insectes phytophages a été largement documenté chez les adultes mais reste peu étudié chez les larves. Les quelques études qui s'y sont intéressées ont montré qu'à l'instar des adultes, les larves étaient capables de s'orienter de manière sélective vers des stimuli olfactifs émis par leurs plantes hôtes (Piesik *et al.*, 2013, 2018). Certaines études ont même pu montrer qu'elles pouvaient, de manière innée, distinguer les odeurs de plantes hôtes de celles de plantes non hôtes (Piesik *et al.*, 2009). Dans certains cas, les signaux volatils utilisés par les femelles et les larves pour reconnaître leur hôte sont identiques. Ainsi par exemple, chez le doryphore (*Leptinotarsa decemlineata*), un mélange de linalool, salicylate de méthyle et d'acétate de cis-hex-3-ènyle émis par les pommes de terre (*Solanum tuberosum*) induit une attraction à la fois des larves et des adultes (Dickens, 2002). Il en est de même chez le carpocapse des pommes et des poires, *Cydia pomonella*, avec le (2E,4Z)-2,4-décadiénoates d'alkyle, un composé volatil émis par les poires Bartlett mûres (*Pyrus communis*) (Knight et Light, 2001 ; Light *et al.*, 2001). Cependant, même lorsqu'ils partagent la même ressource alimentaire, les signaux utilisés peuvent être différents entre les adultes et les larves (Dweck *et al.*, 2018).

En plus des composés volatils, les larves détectent les composés présents à la surface et dans les tissus végétaux grâce à leurs sensilles gustatives (Schoonhoven, 1987). Ces sensilles permettent également dans certains cas de percevoir aussi quelques composés volatils, même si les exemples restent plus rares (Städler et Hanson, 1975 ; Dethier et Goldrich Rachman, 1976 ; Mitchell et McCashin, 1994) – suggérant ainsi que la distinction entre récepteurs sensoriels olfactifs et gustatifs n'est pas si claire et représente plutôt un continuum, avec certains récepteurs qui ne réagissent qu'aux odeurs et sont olfactifs, certains qui ne réagissent qu'aux molécules en solution et sont gustatifs, et certains qui réagissent aux deux et qui ont donc une fonction mixte. Comme dit plus haut dans l'introduction, les neurones associés à ces récepteurs sensoriels sont classés en deux catégories : les cellules phagostimulatrices et les cellules dissuasives. C'est l'activité de ces neurones, en réponse à des stimuli qu'ils reconnaissent spécifiquement, qui stimule ou inhibe l'alimentation. Les métabolites primaires comme les sucres activent très souvent des cellules phagostimulatrices et sont par conséquent connus pour avoir un fort effet stimulant (Schoonhoven, 1987 ; Chapman, 2003), donc favorable à l'acceptation de l'hôte. Les neurones récepteurs peuvent également répondre à des composés spécialisés plus spécifiques à l'hôte, ce qui permettrait aux larves de disposer d'indices plus spécifiques que les métabolites primaires pour discriminer leurs hôtes. Ainsi, il a pu être montré la présence chez huit espèces de chenilles qui se

nourrissent principalement ou uniquement de Rosaceae, d'un neurone qui répond au sorbitol, un composé caractéristique des Rosaceae (Schoonhoven *et al.*, 2002). Sur la base des composés chimiques qu'elles détectent, les larves peuvent donc accepter ou rejeter une plante et donc être les ultimes décisionnaires de la sélection de l'hôte.

4. Le rôle des mères

La sélection de l'hôte chez les insectes phytophages est majoritairement assurée par les adultes, particulièrement les femelles qui choisissent là où elles pondent leurs œufs, donc là où se développeront leurs descendants. Le choix d'un site de ponte représente un aspect majeur de la spécialisation à la plante hôte. En effet, chez la majorité des espèces phytophages, les larves ont une capacité de dispersion et des réserves énergétiques faibles (Jaenike, 1990 ; Thompson, 1988). La quantité et la qualité de la nourriture disponible pour leur développement et leur survie résultent ainsi directement du choix de la mère au moment de la ponte (Gripenberg *et al.*, 2010). La femelle a donc intérêt à sélectionner une plante hôte de la meilleure qualité possible afin d'assurer à sa descendance une performance optimale. Cette idée intuitive a été théorisée sous la forme de l'hypothèse dite de préférence-performance ("*preference-performance hypothesis*" ou PPH, Wiklund, 1975). Cette hypothèse, également connue sous le nom d'*"optimal oviposition theory*" (Jaenike 1978) ou encore "*mother knows best*" (Valladares et Lawton 1991), est basée sur la capacité des femelles à discriminer et classer les plantes hôtes selon des critères de préférence permettant de maximiser la performance des descendants. Un certain nombre d'études ont en effet montré qu'il existait une corrélation positive entre les préférences de ponte des femelles et la performance de leurs larves (Keeler et Chew, 2008 ; Zhang *et al.*, 2012 ; Du *et al.*, 2016). Cependant, beaucoup d'autres ne l'ont pas mise en évidence (Mayhew 1997, 2001 ; Jones *et al.*, 2019). Plusieurs éléments sont susceptibles d'expliquer cette absence de corrélation. D'une part, les larves peuvent être capables de localiser elles-mêmes un hôte approprié, de sorte que le site de ponte peut ne pas être un déterminant majeur de la performance larvaire (Cunningham *et al.*, 2011 ; Rivera et Burrack, 2012). D'autre part, les femelles peuvent choisir des plantes qui maximisent leurs propres longévité et fécondité (Scheirs *et al.*, 2000). En effet, lorsque la meilleure nourriture pour les femelles diffère de celle pour les larves, la quantité de descendants supplémentaires gagnée via le choix de la nourriture la plus favorable aux femelles peut être suffisante pour compenser les coûts liés à la réduction du nombre et de la qualité des descendants produits. Cette stratégie de sélection de la plante hôte, qui peut être adaptative sous certaines conditions, illustre le compromis évolutif entre reproduction immédiate et différée auquel les femelles font face.

III. Être un insecte souterrain, qu'est-ce que ça change dans la recherche de la plante hôte ?

Bien que les insectes souterrains soient généralement moins bien étudiés que leurs homologues aériens (Hunter, 2001), il est largement reconnu qu'ils jouent un rôle central dans les écosystèmes terrestres (Johnson et Murray, 2008). Contrairement aux insectes aériens qui peuvent exploiter des signaux visuels des plantes, les insectes souterrains ne peuvent se fier qu'aux indices tactiles et chimiques pour rechercher et sélectionner leur hôte.

Un certain nombre d'études comportementales ont montré que les herbivores souterrains étaient capables de détecter la présence des plantes hôtes en amont de tout contact physique (Johnson *et al.*, 2004). Les insectes souterrains possèdent en effet des sensilles olfactives et gustatives (Eilers *et al.*, 2012 ; Weissteiner *et al.*, 2012), mais la distinction entre les exsudats volatils et solubles est beaucoup plus floue dans la rhizosphère qu'en milieu aérien (Erb *et al.*, 2013). En effet, les COV sont susceptibles de diffuser dans le sol à l'interface entre la phase liquide et la phase gazeuse. D'autre part, certains composés solubles peuvent être perçus comme des volatils (Bjostad et Hibbard, 1992), et il a même pu être montré que les sensilles gustatives des larves de hanneton, *Melolontha melolontha*, pouvaient percevoir à la fois des molécules gazeuses et solubles (Eilers *et al.*, 2012). En plus du CO_2 **[Encart 3]**, environ 74 composés exsudés par les racines ont été reportés dans la littérature comme ayant une influence sur le comportement des phytophages souterrains, dont une grande majorité (>80 %) a des effets attractifs et les autres des effets répulsifs ou attractif/répulsif selon leur concentration et l'espèce d'insecte (Johnson et Gregory, 2006 ; Johnson et Nielsen, 2012). Parmi les composés identifiés, un certain nombre de métabolites spécialisés spécifiques à certaines plantes hôtes induisent une attraction des insectes spécialistes de ces plantes (Matsumoto et Thorsteinson, 1968 ; Soni et Finch, 1979). Dans leurs synthèses, Johnson et Gregory (2006) et Johnson et Nielsen (2012) ont recensé seulement deux composés ayant un effet sur plus de deux espèces et plusieurs auteurs (Jones et Coaker, 1978 ; Ross et Anderson, 1992) ont suggéré que la spécificité des exsudats chimiques spécialisés serait à l'origine de la distinction entre plantes hôtes et non hôtes chez les insectes souterrains, particulièrement chez les spécialistes. Comme les insectes aériens, les souterrains auraient évolué de façon à utiliser les composés spécialisés, servant à l'origine de défense pour les plantes, comme signaux spécifiques pour reconnaître leur hôte. Un exemple marquant de ceci est celui des larves de la mouche de la carotte, *Psilae rosae* qui sont attirées par le falcarinol, un composé pourtant neurotoxique mais produit par leur plante hôte, la carotte (*Daucus carota*) (Maki *et al.*, 1989).

En plus des exsudats racinaires, d'autres composés chimiques peuvent influencer le comportement alimentaire des phytophages souterrains lorsqu'ils entrent en contact physique avec les racines. Ce sont les composés situés à la surface des racines et ceux internes aux tissus racinaires. La majorité des

composés reconnus comme phagostimulants sont des métabolites primaires comme des acides aminés et surtout des sucres (>50 % des phagostimulants identifiés), tandis que la plupart des composés dissuasifs sont des métabolites spécialisés (Erb *et al.*, 2013). Bien que la majorité des composés phagostimulants identifiés soient des métabolites primaires, et donc communs à un très grand nombre de plantes, Johnson et Gregory (2006) suggèrent que, comme en milieu aérien, la combinaison de ces composés peut être importante dans la reconnaissance de l'hôte. Quelques rares études ont mis en évidence l'effet phagostimulant des métabolites spécialisés sur des larves souterraines spécialistes. C'est le cas notamment des benzoxazinoïdes, des phagostimulants pour la chrysomèle du maïs, *Diabrotica virgifera virgifera* (Robert *et al.*, 2012). De même, Deheer et Tallamy (1991) ont constaté que l'alimentation de la chrysomèle tachetée du concombre, *Diabrotica balteata*, était stimulée par des cucurbitacines, pourtant amères et toxiques.

Encart 3 Le rôle controversé du CO_2

Comme chez les insectes aériens, les herbivores souterrains sont capables d'utiliser des composés volatils pour localiser une plante. Du fait de son abondance dans le milieu souterrain, causée par les émissions respiratoires des plantes, le CO_2 est l'exsudat racinaire le plus largement signalé comme impliqué dans l'attraction d'herbivores racinaires (Johnson et Nielsen, 2012 ; Johnson et Gregory, 2006 ; Doane *et al.*, 1975) et inversement, de très fortes concentrations peuvent les repousser (Bernklau et Bjostad, 1998). Cependant, les émissions de CO_2 par les racines étant omniprésentes dans le sol, elles ne représentent pas un indice chimique clé qui pourrait permettre aux insectes et particulièrement aux spécialistes de distinguer leur hôte. Ces constats ont conduit à émettre l'hypothèse suivante : les insectes ayant une très large gamme d'hôtes pourraient utiliser le CO_2 comme indice pour localiser leur hôte alors que ceux ayant une gamme plus étroite devraient se fier aux exsudats racinaires qui sont plus spécifiques à la plante hôte (Johnson et Gregory, 2006).

IV. Modèle biologique

1. Les plantes hôtes exploitées

La famille des Brassicaceae (anciennement Crucifères) appartient à l'ordre des Brassicales et se compose de 351 genres pour 3 977 espèces sauvages ou cultivées (Brassibase). La particularité des Brassicaceae réside dans leur grande diversité de profils chimiques essentiellement liée à la production de métabolites spécialisés. En effet, les glucosinolates (GLS), aussi connus sous le nom de thioglucosides, constituent un groupe majeur de composés soufrés non volatils présent dans presque toutes les espèces de la famille des Brassicaceae (Bhandari *et al.*, 2015). Un minimum de 120 GLS différents ont été identifiés (Fahey *et al.*, 2001). Ces composés sont stockés dans des vacuoles au sein de cellules non spécifiques (Grob et Matile, 1979) et, lorsque les tissus de la plante sont endommagés (suite par exemple à leur consommation par un phytophage broyeur), ils entrent en contact avec l'enzyme *β*-thioglucoside (myrosinase) séquestrée dans les grains de myrosine au sein de cellules spécifiques, ce qui va déclencher leur hydrolyse (Lüthy et Matile, 1984). Les composés toxiques issus de cette hydrolyse, appelés isothiocyanates (ITC), sont également des composés soufrés mais sont volatils, contrairement aux GLS.

Il existe d'importantes variations quantitatives et qualitatives des GLS entre les espèces de Brassicaceae (Griffith *et al.*, 2001), dont chacune ne contient généralement qu'un nombre limité de ces composés parmi la centaine identifiée. Au sein d'une même espèce, une variation considérable des profils de GLS peut également être observée. Par exemple, Kliebenstein *et al.* (2001) ont mis en évidence une variation qualitative et quantitative dans la composition de 34 GLS différents lors de l'analyse des feuilles et des graines de 39 écotypes d'*Arabidopsis thaliana*. Des variations de niveaux de GLS sont également observées au sein d'une même plante. À travers l'analyse de 74 études sur les GLS constitutifs des racines et des feuilles de 29 espèces végétales, van Dam *et al.* (2009) ont pu montrer que, d'une manière générale, les racines présentaient des concentrations plus élevées et une plus grande diversité de GLS que les feuilles. La spécificité de ces profils à la fois entre les espèces, entre les populations et au sein d'un même individu peut donc donner des indices très précis aux insectes phytophages, leur permettant ainsi de reconnaître leur plante hôte. Leur effet sur la stimulation de l'oviposition et de l'alimentation a de fait été mis en évidence chez plus de 25 espèces de Diptères, Coléoptères et Lépidoptères phytophages spécialistes des Brassicaceae (Hopkins *et al.*, 2009).

2. Le phytophage *Delia radicum*

A. Généralités et cycle biologique

La mouche du chou, *Delia radicum*, est un diptère phytophage de la famille des Anthomydiiae. Dans la littérature, plusieurs synonymes désignant cette espèce peuvent être rencontrés : *Delia brassicae* (Wiedemann), *Chortophila brassicae* (Bouché), *Hylemya brassicae* (Bouché) ou encore *Erioischia brassicae* (Bouché) (Finch, 1978 ; Brittain, 1923 ; Mukerji et Harcourt, 1970 ; Traynier, 1967a). Cet insecte est présent dans tout le nord de la région holarctique, de l'Amérique du Nord à l'Asie en passant par l'Europe et l'Afrique. Il est originaire de la région paléarctique et aurait été introduit en Amérique du Nord au XIXe siècle (Biron *et al.*, 2000). Son cycle de développement est d'environ 30 jours en conditions climatiques optimales. Les femelles pondent leurs œufs au collet des plantes et, lorsque les œufs éclosent, les larves qui en sortent se dirigent ensuite dans le sol afin de se nourrir des racines – causant d'ailleurs d'importants dégâts dans les cultures de Brassicaceae de tout l'hémisphère Nord (King et Forbes, 1954 ; Biron *et al.*, 2000). La phase larvaire se compose de trois stades et la nymphose à lieu dans le sol à proximité des racines. L'imago met environ 10 jours avant d'émerger (Fig. 4). Il s'agit d'une espèce à la fois aérienne et souterraine, et la femelle sélectionne la plante sur laquelle déposer ses œufs en milieu aérien tandis que la larve complète son cycle de développement en exploitant la partie souterraine de la plante.

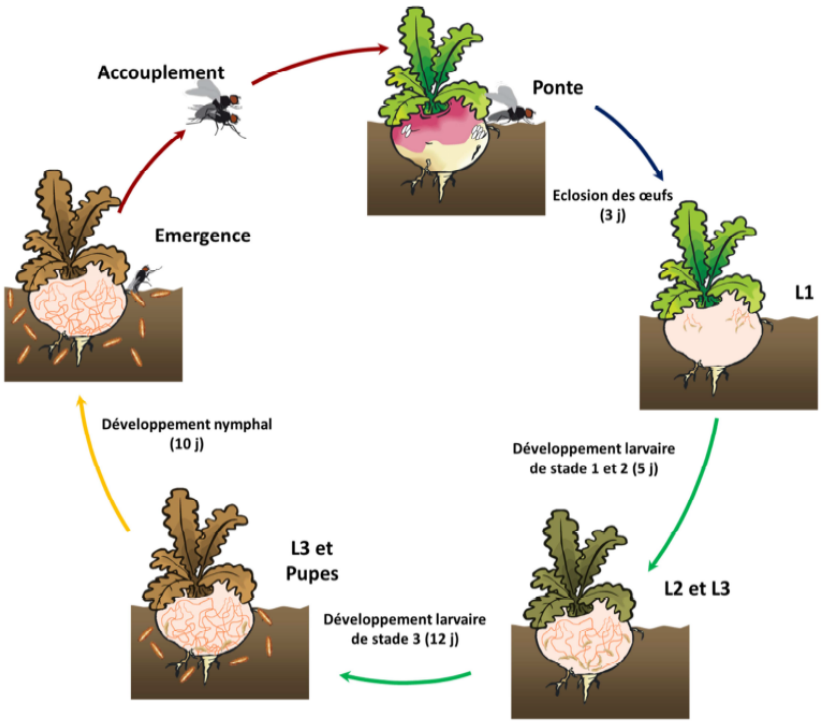


Figure 4. Cycle biologique de *Delia radicum* et durée des différents stades en conditions contrôlées à 20° (Lamy, 2016).

B. La spécialisation pour l'oviposition des femelles aériennes

Les adultes de *D. radicum* sont généralistes pour l'alimentation et se nourrissent sur les fleurs de nombreuses espèces de plantes (Ceasar, 1922 ; Finch et Coaker, 1969). Après l'émergence et pendant les deux à trois premiers jours de leur vie, les adultes non accouplés sont à la recherche de sources de nourriture (Finch et Coaker, 1969a). Ils se nourrissent du nectar de nombreuses espèces de plantes comme par exemple les Rosacées, les Ombellifères ou les Lamiacées (Finch et Coaker, 1969b). Un manque de protéines et de sucres dans l'alimentation des femelles réduit leur fécondité et leur longévité (Košťál, 1993), soulignant ainsi l'importance de l'alimentation dans la maturation ovarienne chez les femelles. D'autre part, cette étude a également montré que l'absence d'une plante hôte à la disposition des femelles entraînait un blocage de l'oviposition ainsi qu'un retard de développement des follicules ovariens. Il est donc important pour les femelles de se nourrir puis de localiser une plante hôte sur laquelle déposer ses œufs si elle veut assurer sa reproduction.

Les femelles sont oligophages, spécialistes des Brassicaceae pour la ponte (Capinera, 2008). Des observations sur le terrain ont mis en évidence leurs déplacements entre une culture de Brassicaceae (site de ponte) et les haies environnantes où se trouvent les fleurs (site d'alimentation) (Hawkes, 1972). Si le site d'alimentation est dissocié du site de ponte, alors les femelles doivent être capables, après s'être alimentées, de localiser à distance un hôte et de s'en approcher pour éventuellement y déposer leurs œufs. Pour cela, elles utilisent à la fois des signaux physiques et chimiques produits et émis par les plantes. Les stimuli physiques comme la couleur, la forme ou la taille de la plante, bien qu'importants pour la recherche à distance de l'hôte (Prokopy *et al.*, 1983 ; Tuttle *et al.*, 1988 ; Brown et Anderson, 1996), restent moins spécifiques que les médiateurs chimiques. Au sein des Brassicaceae, les femelles ont des préférences pour certaines espèces ou certaines populations (Doane et Chapman, 1962 ; Radcliffe et Chapman, 1966 ; Ellis et Hardman, 1975 ; Rousse *et al.*, 2003 ; Lamy *et al.*, 2020), suggérant la capacité de discriminer les différents hôtes grâce à leur profil chimique.

C. Les signaux utilisés pour la recherche à distance

Les femelles possèdent sur leurs antennes des sensilles olfactives qui leur permettent de percevoir les COV (Ross et Anderson, 1987, 1991a ; Ross, 1992) (Fig. 5). Le rôle des bouquets d'odeurs émis par les plantes dans l'orientation à distance des femelles a été démontré (Traynier, 1967b ; Finch, 1978 ; Hawkes *et al.*, 1978 ; Hawkes et Coaker, 1979 ; Finch et Skinner, 1982 ; Nottingham, 1987). Dans leur étude, Hawkes et Coaker (1979) ont constaté que seules les femelles gravides montraient une réponse olfactive orientée, renforçant l'idée de l'implication des COV dans le processus de sélection de l'hôte. Des contrastes d'attractivité entre différentes espèces et différentes populations de plantes hôtes ont été observés, suggérant des capacités de discriminations fines des signaux volatils perçus par les femelles

(Kergunteuil *et al.*, 2015a ; 2015b). Des composés caractéristiques des Brassicaceae comme les ITC, en particulier l'isothiocyanate d'allyle, sont attractifs pour les femelles (Hawkes *et al.*, 1978 ; Finch, 1978 ; Hawkes et Coaker, 1979 ; Wallbank et Wheatley, 1979 ; Finch et Skinner, 1982 ; Nottingham et Coaker, 1985, 1987 ; Tuttle *et al.*, 1988). D'autres composés moins spécifiques comme le linalool, le *β*-caryophyllène ou l'*α*-farnésène pourraient également être impliqués dans l'attraction à distance (Kergunteuil *et al.*, 2015a). D'après Städler (1978) et de Jong et Städler (1999), les COV seuls ne suffiraient pas à induire la ponte mais pourraient agir en synergie avec des composés de contact pour la stimuler.

D. Les signaux utilisés pour l'évaluation au contact

Après s'être posées sur un hôte potentiel, les femelles explorent la surface de la plante à travers un comportement très stéréotypé (Zohren, 1968) (Fig. 7 pour une représentation visuelle schématique). Elles entrent alors en contact avec les composés de surface, sur la base desquels elles vont accepter l'hôte ou non. L'extraction des composés de surface d'espèces de Brassicaceae comme l'arabette (*Arabidopsis thaliana*) (Marazzi et Städler 2004), le colza (*Brassica napus*) ou le chou (*Brassica oleracea*) (Baur *et al.*, 1996, 1998) et leur test sur le comportement d'oviposition des femelles a permis de montrer qu'ils étaient impliqués dans la stimulation de la ponte. Les GLS présents dans les extraits de surface foliaire stimulent l'oviposition (Hardman et Ellis 1978 ; Roessing *et al.*, 1992 ; Braven *et al.*, 1996 ; Chilcott 1997 ; Baur *et al.*, 1998 ; Griffiths *et al.*, 2001 ; Marazzi et Städler 2004 ; Gouinguené et Städler 2006) et sont supposés être fortement impliqués dans la sélection de l'hôte par les femelles. En plus des GLS, d'autres composés appelés '*Cabbage Identification Factors*' (CIF) et présents à la surface des feuilles de Brassicaceae (Roessingh *et al.*, 1992 ; Roessingh *et al.*, 1997 ; Hurter *et al.*, 1999) stimulent fortement la ponte des femelles (Baur *et al.*, 1996 ; de Jong *et al.*, 2000 ; Gouinguené et Städler, 2006). Les récepteurs gustatifs situés sur les tarses des femelles (Fig. 5) détectent ces composés de surface et y sont sensibles, même à de très faibles doses (Städler 1978 ; Isidoro *et al.*, 1994 ; Roessingh *et al.*, 1997 ; Marazzi et Städler 2004 ; Gouinguené et Städler, 2005, 2006). D'autres composés comme le disulfure de diméthyle (DMDS) ou le toluquinone ont plutôt un effet dissuasif et réduisent l'oviposition (Den Ouden *et al.*, 1996 ; Kergunteuil *et al.*, 2012 ; Lamy *et al.*, 2017, 2018)

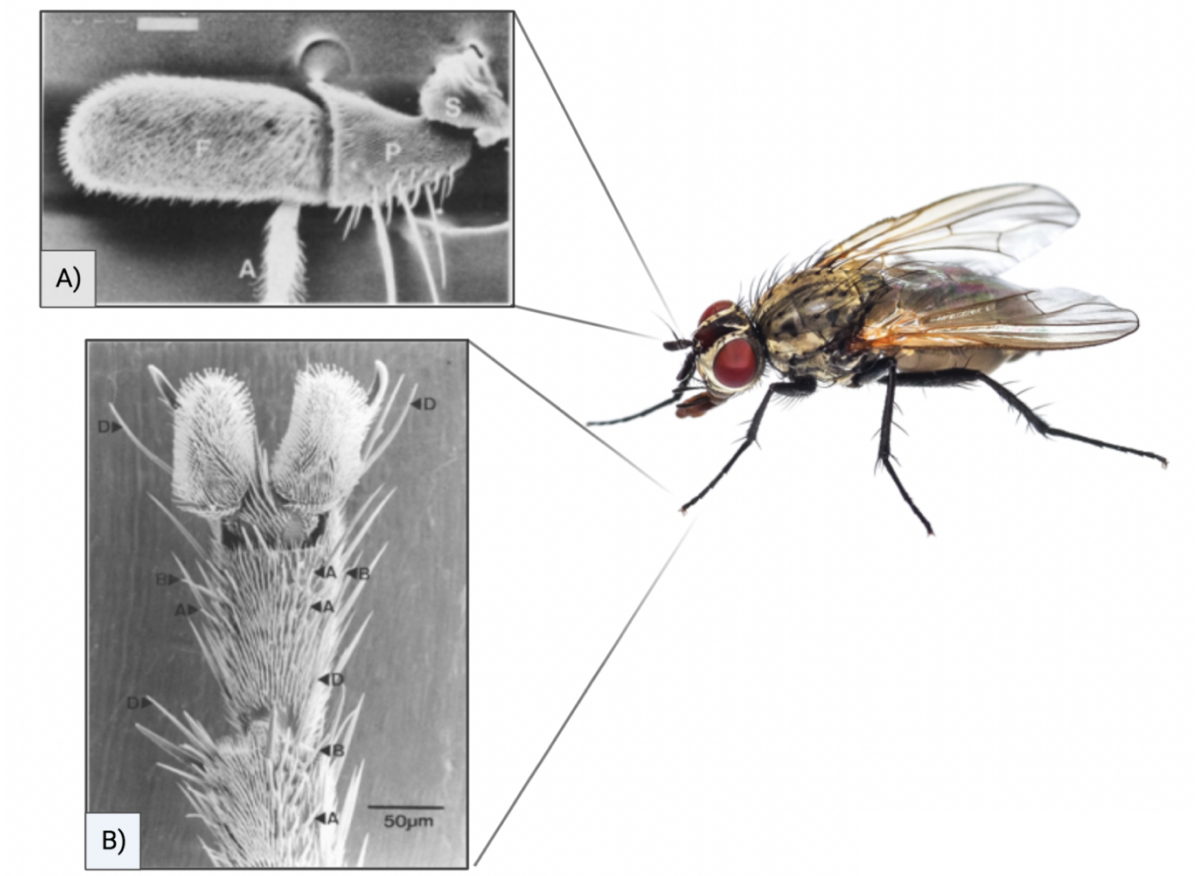


Figure 5 : Photographie en microscopie électronique à balayage A) de la face dorsale d'une antenne et des différentes structures qui la composent (A = arista, F = funicule, P = pédicelle, S = scape) (d'après Ross et Anderson, 1987) et B) de la face ventrale de l'extrémité du tarse de la patte prothoracique d'une femelle *D. radicum* (A à D : sensilles réceptrices tarsales) (adapté d'après Städler, 1978)

E. Les signaux utilisés par les larves souterraines

Comme chez la plupart des insectes phytophages, les études sur les signaux utilisés par les larves lors de la sélection de l'hôte sont beaucoup plus rares que celles sur les adultes. Certaines ont tout de même montré que l'attraction des larves était stimulée par les odeurs de plantes hôtes, et qu'elles étaient repoussées (ou non attirées) par les odeurs de plantes non hôtes (Ross et Anderson 1992). Un certain nombre de COV ont une influence sur la localisation de l'hôte chez la larve comme certains ITC dont l'isothiocyanate d'allyle (Košťál, 1992 ; Ross et Anderson, 1992). D'autres composés plus communs comme l'acétate de cis-hex-3-ènyle (un « composé vert », dérivé d'acides gras) ou certains terpènes ont un effet répulsif sur les larves (Košťál, 1992). Les larves sont capables de détecter les composés volatils et les exsudats racinaires grâce à leurs sensilles olfactives et gustatives situées sur les lobes céphaliques (Ryan et Behan 1973 ; Ross et Anderson, 1991b) (Fig. 6). Le succès de développement des larves est très variable d'une espèce et d'une population hôte à l'autre (Doane et Chapman, 1962 ; Ellis *et al.*, 1999 ; Felkl *et al.*, 2005 ; Finch et Ackley, 1977 ; Hopkins *et al.*, 1999 ; Shuhang *et al.*, 2016).

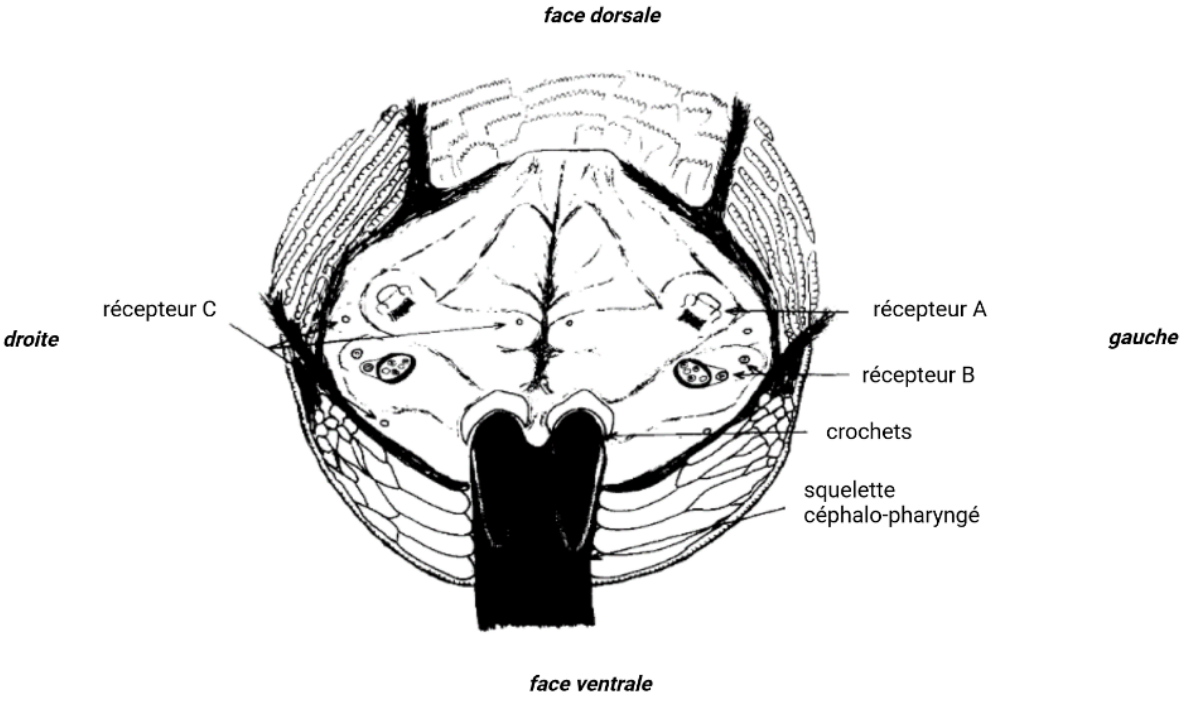


Figure 6 : Schéma de la vue externe de la région antérieure (vue frontale) de la larve de *D. radicum* montrant la distribution des récepteurs sensoriels sur les lobes céphaliques. Récepteur A : chimiorécepteur olfactif ; récepteur B : chimiorécepteurs olfactifs et gustatif ; récepteur C : mécanorécepteurs (issu de Ryan et Behan, 1973).

V. Objectifs de la thèse et présentation des articles

Comme nous l'avons vu dans l'introduction, les femelles adultes jouent un rôle important en choisissant le site de ponte mais les larves, même si elles sont souterraines, peuvent également être impliquées dans l'acceptation ou non de la plante. De fait, le processus de sélection de la plante hôte ne repose pas uniquement sur le choix des femelles. Étudier les signaux impliqués à la fois dans la sélection par les adultes et par les larves semble donc nécessaire pour tirer des conclusions plus globales sur les mécanismes impliqués dans la sélection de l'hôte chez les insectes phytophages. Nous avons également vu que les différentes phases comportementales de la sélection de l'hôte, que ce soit chez les adultes ou chez les larves, sont en grande partie médiées par les signaux chimiques des plantes et que la nature des signaux chimiques à l'origine de la discrimination d'un hôte face à un non hôte est largement décrite. Cependant, bien que des préférences inter- et intraspécifiques au sein d'une même gamme d'hôtes soient observées pour de nombreux insectes phytophages, l'implication exacte des signaux chimiques dans ces préférences est moins bien comprise. En effet, les études se focalisant à la fois sur plusieurs espèces et plusieurs populations restent minoritaires, alors que la combinaison de ces deux échelles permet de comprendre de façon plus détaillée à la fois les signaux permettant à l'insecte de discriminer différentes espèces d'hôtes et les signaux à l'origine de ses préférences au sein d'une même espèce. Le travail effectué au cours de cette thèse avait donc pour objectif principal de **décrypter les mécanismes impliqués dans l'acceptation de l'hôte et les préférences inter- et intraspécifiques des larves et des adultes chez un insecte phytophage spécialiste**.

Pour répondre à cet objectif, nous avons développé une démarche générale consistant d'abord à isoler les comportements correspondant à chaque étape de l'interaction (recherche à distance et évaluation au contact), afin de pouvoir examiner l'effet des différents signaux chimiques de la plante sur chacune de ces étapes (Fig. 7). Pour ce faire, nous avons combiné des expérimentations sur plantes entières et sur substrat artificiel sur lequel étaient déposés les extraits de plantes. Afin d'appréhender les différents signaux à l'origine des préférences des insectes, trois espèces hôtes et plusieurs populations (ici des cultivars) ont systématiquement été testés, dans le but de couvrir un minimum de diversité inter- et intraspécifique (Tab. 1). Toutes les études de cette thèse ont été réalisées en laboratoire, sur une population de mouche du chou et des populations de différentes espèces de Brassicaceae commerciales (Tab. 1) respectivement élevées et cultivées en conditions contrôlées.

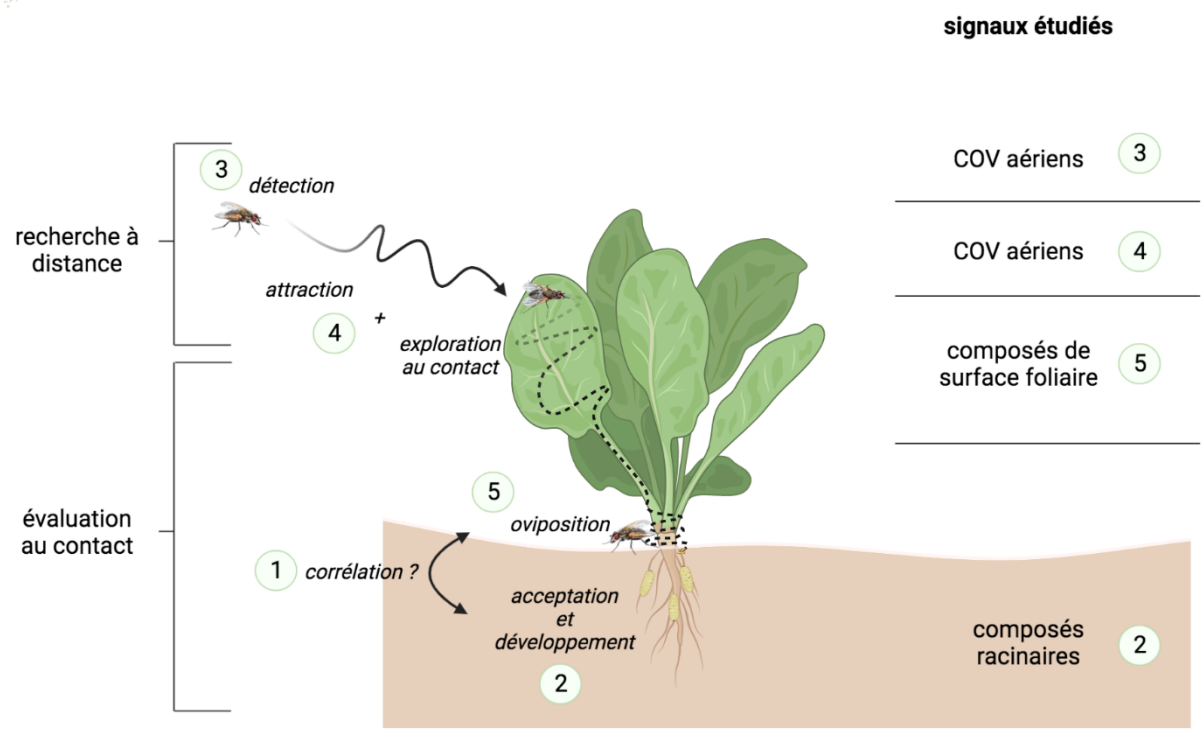


Figure 7. Les différentes étapes de la sélection de la plante hôte chez *D. radicum* étudiées au cours de cette thèse, ainsi que les signaux sur lesquels se focalisent les différents chapitres du manuscrit. Des numéros indiquent les chapitres dans lesquels sont abordés les différents mécanismes (à gauche) et les signaux correspondant (à droite).

Tableau 1 : Diversité inter et intraspécifique choisie pour les différents travaux de la thèse.

Espèce	**Sous-espèce**	**Population**
Brassica oleracea	*italica*	Parthenon
Brassica rapa	*pekinensis*	Richi Storkin Tabaluga Yuki
Sinapis alba		Brillant Cezara Sarah Verte

Le travail réalisé au cours de cette thèse est présenté sous forme d'articles scientifiques publiés ou en préparation formant les cinq parties de ce manuscrit.

Article 1 : Challenging the PPH in an above-belowground insect

⟶ Publié dans *Oecologia*

L'objectif de cette étude était d'établir la relation entre les préférences de ponte des femelles et la performance des descendants au sein d'une même gamme de plantes hôtes, et de tester s'il existe des contrastes entre trois espèces (*Brassica oleracea*, *B. rapa* et *Sinapis alba*) et quatre populations au sein de *B. rapa* et *S. alba*. Elle a permis de tester l'hypothèse de préférence-performance (PPH) sur un modèle dont les adultes sont aériens et les larves souterraines, ce qui est rarement fait dans la littérature qui se concentre essentiellement sur les phytophages entièrement aériens. Des expériences de développement larvaire et d'oviposition sur plantes entières ont été réalisées pour répondre à cet objectif. Les résultats montrent d'une part que suivant la variable utilisée pour estimer la performance larvaire (temps de développement, taille des descendants, temps de survie après l'émergence), une corrélation positive entre préférence des femelles et performance larvaire peut être retrouvée ou non. De plus, l'existence d'une corrélation positive au sein de *B. rapa*, mais négative au sein de *S. alba* et absente à l'échelle interspécifique, montre l'importance d'inclure différentes échelles d'observation avant d'émettre des conclusions générales sur la validation de la PPH chez les insectes phytophages.

Article 2 : Root compounds mediate host acceptance in larvae of an above-belowground specialist phytophagous insect

⟶ Manuscrit en préparation

L'absence de corrélation entre le choix des femelles et la performance des larves au sein d'une des espèces étudiées dans l'article 1 nous a conduit à évaluer l'implication des composés racinaires dans l'acceptation de l'hôte par les larves, afin de déterminer si les différentiels de performance larvaire étaient le reflet d'un différentiel d'acceptation. Pour cela une première expérience sur plante entière a été menée dans laquelle les systèmes racinaires de plantes infestées ou non par des larves ont été mesurés après leur développement afin de déterminer si les larves consommaient toutes les plantes testées. Puis, des tests d'alimentation sur substrats artificiels supplémentés en extraits racinaires totaux des différentes espèces et cultivars ont été réalisés. Enfin, une approche de fractionnement bioguidé a été suivie dans l'objectif de réduire le nombre de composés candidats potentiellement impliqués dans l'acceptation (ou le rejet) de la plante par les larves.

Des analyses métabolomiques sont en cours sur la sous-fraction identifiée comme responsable du contraste d'alimentation entre les deux populations de *S. alba*, pour proposer une liste de composés candidats potentiellement impliqués dans ce contraste. Pour *B. rapa*, des effets de deux combinaisons de fractions ont été identifiés comme impliqués dans la stimulation de l'alimentation mais le sous-fractionnement n'a pas encore été réalisé.

Article 3 : Sex- and maturity-dependent antennal detection of host plant volatiles in the cabbage root fly, *Delia radicum*

⟶ Manuscrit prêt à la soumission dans *Journal of Insect Physiology*

Plusieurs études ont pu montrer que les femelles gravides de *D. radicum* répondaient significativement plus au COV émis par leurs hôtes contrairement aux femelles non gravides, suggérant des changements de réponse dans la détection antennaire des odeurs en fonction de la maturité sexuelle. Malgré ces observations, la détection antennaire des COV selon la maturité reste peu étudiée chez *D. radicum*. De plus, beaucoup d'études se focalisent sur des composés spécifiques mais très peu sur des composés ubiquistes. Les connaissances sur l'utilisation des COV par les mâles sont également très limitées. L'objectif de cette expérimentation était d'étudier si la détection antennaire de composés volatils clés identifiés à partir de plantes hôtes, ubiquistes et spécifiques, est influencée par le sexe ou la maturité sexuelle, ce qui pourrait expliquer les différences dans le comportement guidé par les odeurs chez *D. radicum*. Pour cela, les réponses des antennes à 15 COV émis par différentes Brassicaceae et sélectionnés pour leur diversité chimique ont été étudiées, par enregistrements électro-antennographiques.

Article 4 : Host plant selection in phytophagous insects: volatiles can act independently at distance and at contact

⟶ Manuscrit prêt à la soumission dans *Oecologia*

L'implication des COV dans l'orientation et la recherche à distance de l'hôte chez les femelles *D. radicum*, ainsi que la composition des bouquets d'odeur émis par différentes Brassicaceae, ont largement été décrits dans la littérature. Leur effet sur la stimulation de l'oviposition, en synergie avec des composés de surface, a également été rapporté. Cependant, l'effet des COV sur l'initiation des comportements d'oviposition n'a jamais été testé, bien que cela permettrait de mieux comprendre leur implication dans le processus de sélection de l'hôte par les femelles. Ainsi, l'objectif de cette étude était double. Dans un premier temps, elle visait à identifier les composés qui à la fois sont présents dans le bouquet de COV émis par différents hôtes et sont détectés par les femelles, afin de distinguer quelle partie du mélange volatil pouvait avoir un effet sur la reconnaissance de l'hôte. Pour cela, des captures

d'odeurs ont été réalisées puis la composition des bouquets a été analysée par chromatographie en phase gazeuse couplée à de la spectrométrie de masse (GC-MS). Les composés perçus par les femelles ont été identifiés par électrophysiologie (chromatographie en phase gazeuse couplée à une détection électro-antennographique et un détecteur à ionisation de flamme) (GC-EAD/GC-FID). Dans un second temps, cette étude visait à tester l'effet des différents composés éléctrophysiologiquement actifs sur le comportement des femelles, en utilisant un dispositif de feuille artificielle et des composés volatils synthétiques. Des observations comportementales ont été réalisées afin de voir si les bouquets de COV testés attiraient les femelles sur les feuilles artificielles, les stimulaient à explorer la surface de ces feuilles une fois à leur contact, ou bien les deux. L'objectif de ces tests comportementaux était de distinguer les effets des COV à courte distance des effets au contact (ce que ne permettent pas des études comportementales d'olfactométrie), afin de mieux comprendre leur implication dans les différentes phases de la séquence comportementale du choix de l'hôte.

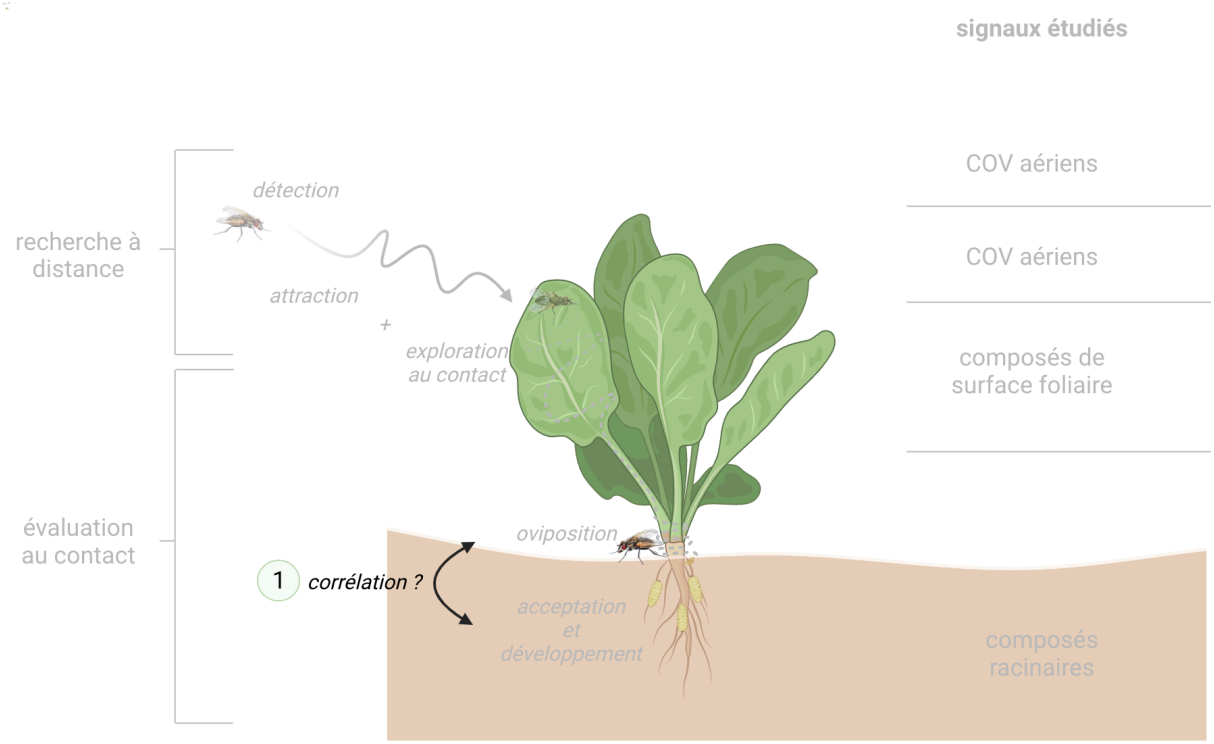
Article 5 : Host recognition mechanisms can vary within the host range of a phytophagous insect: oviposition is not always triggered by leaf surface compounds

⟶ Manuscrit prêt à la soumission dans *Oecologia*

De nombreuses études ont démontré l'implication des composés de surface foliaire dans la stimulation de l'oviposition chez *D. radicum*, mais un grand nombre d'entre elles a travaillé sur une seule espèce de plante. Il est cependant bien connu que les femelles possèdent des préférences à l'échelle inter- et intraspécifique. De plus, une seule étude a examiné l'implication de ces composés dans les préférences aux deux échelles. L'article 1 nous a permis de clarifier les préférences d'oviposition des femelles, mais n'a pas visé à identifier les signaux responsables de ces préférences. L'objectif de cette étude était d'évaluer l'implication des composés de surface dans les préférences inter- et intraspécifique d'oviposition chez *D. radicum*, afin de déterminer s'ils servent de signaux aux femelles pour discriminer leurs différents hôtes. Des expériences de ponte sur feuilles artificielles supplémentées en extrait de surface des feuilles de différents hôtes contrastés pour l'oviposition ont été réalisées

Article 1

Challenging the Preference-Performance Hypothesis in an above-below ground insect



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PLANT-MICROBE-ANIMAL INTERACTIONS – ORIGINAL RESEARCH

Challenging the Preference–Performance Hypothesis in an above-belowground insect

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Abstract
The relationship between female oviposition preference and offspring performance has been a question of special interest in the study of host plant selection by phytophagous insects. The Preference–Performance Hypothesis (PPH) is one of the main hypotheses proposed to explain this relationship, stating that females should preferentially lay eggs on plants providing the best larval development. The PPH has been extensively tested on aboveground insects but its application to species with belowground larvae is still mostly unknown. In this study, the PPH was quantitatively tested in an above-belowground insect, the cabbage root fly *Delia radicum*. Female oviposition preference and larval performance were estimated on three brassicaceous species (*Brassica oleracea*, *Brassica rapa,* and *Sinapis alba*) as well as between four cultivars of *B. rapa* and four cultivars of *S. alba*. Larval performance was estimated through their survival and through three life-history traits (LHT) of emerging adults. The PPH was supported at the intraspecific scale but only in *B. rapa* and for some, but not all, of the life-history traits. No support for the PPH was found in *S. alba* as well as at the interspecific scale. This study pleads for the integration of insects with both above- and belowground life stages in the preference–performance debate. Moreover, it raises the importance of measuring several variables to estimate larval performance and to test the PPH quantitatively, both at the plant intraspecific and interspecific scales, before drawing general conclusions.

Keywords Plant–insect interactions · Host plant selection · *Delia radicum* · *Brassicaceae*

Introduction

The selection of a host plant where to lay eggs is a key determinant of female fitness in phytophagous insects. Indeed, the host plant greatly influences the growth and development of larvae (Awmack and Leather 2002). The relationship between larval development and the potential preferences of the female for some host plants was widely studied, especially in the context of the 'preference-performance hypothesis' (PPH). This hypothesis, also known as the 'optimal oviposition theory' (Jaenike 1978) or 'mother knows best' theory (Valladares and Lawton 1991), predicts that females maximize their fitness by laying eggs preferentially on plants that maximize their offspring performance. This should be particularly true in species with rather immobile larvae since the performance of these depends greatly on the female host choice (Gripenberg et al. 2010). A number of studies have found a positive correlation between female preference and larval performance as predicted by the PPH, whereas many others have failed to find it (Mayhew 2001; Gripenberg et al. 2010; Jones et al. 2019). One of the reasons why the PPH is not always supported is that both the interspecific (i.e. between plant species) and the intraspecific (i.e. within a given species) scales are rarely assessed in the same study, and meta-analyses usually confound them (Jones et al. 2019). However, host selection can be a different process at the interspecific and at the intraspecific scales, leading to a preference–performance correlation at only one of these scales. It is then crucial to differentiate these two scales before drawing general conclusions, each providing a different level of understanding of the relationship between phytophagous insects and their host plants (Jones et al. 2019).

Despite the broad interest for the PPH, this hypothesis has been tested essentially on aboveground insects (e.g. Zhang et al. 2012; Charlery de la Masselière et al. 2017;

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Altesor and González 2018). Very few studies have focused on species with aboveground adults and belowground larvae, although selective oviposition should be particularly beneficial for these insects which earliest larval stages usually have a very limited mobility (García-Robledo and Horvitz 2012). Since Johnson et al. (2006) suggested including soil-dwelling insects in the preference–performance debate, to our knowledge only three other studies have been published, which do not allow drawing any general conclusion. Moreover, all these studies were at the plant intraspecific scale and performed qualitative assessments of the PPH since only two groups of plants were compared (Table 1). Thus, whether the PPH applies to species with aboveground adult stages and belowground feeding larvae remains largely unknown.

In this study, we conducted a quantitative assessment of the PPH, both at the plant intraspecific and interspecific scales, in the oligophagous cabbage root fly *Delia radicum* L. (Diptera: Anthomyiidae). Adults of *D. radicum* live aboveground and females oviposit near the stem of brassicaceous plants. Once hatched, soil-dwelling larvae feed on plant roots. Host selection by females is partly determined by plant physical cues such as shape, color, and texture (Prokopy et al. 1983; Roessingh and Städler 1990; Košťál 1993). Females also make use of plant chemical cues, which are either volatile (Finch 1978; Nottingham 1988; Tuttle et al. 1988; Ferry et al. 2009; Kergunteuil et al. 2015a, 2015b) or on the surface of the plant (Städler 1978; Roessingh et al. 1997; Städler and Schöni 1990; De Jong et al. 2000). It has long been shown that in *D. radicum*, females have plant intra and interspecific oviposition preferences (Doane and Chapman 1962; Radcliffe and Keith Chapman 1966; Ellis et al. 1999; Lamy et al. 2020). Two studies even showed a relationship between female preference and offspring performance (Rousse et al. 2003; Van Leur et al. 2008). However, these studies were only qualitative and observations were only performed at one plant observation scale, either intraspecific or interspecific. In the present study, experiments were conducted on two different plant species (the white mustard *Sinapis alba* and Chinese cabbage *Brassica rapa* subsp. *pekinensis*) and four cultivars of each species. Cabbage (*Brassica oleracea*) was also added as a reference species since a substantial part of the knowledge on *D. radicum* was acquired on this species. The PPH was tested in controlled conditions using a combination of multiple-choice egg-laying tests and a development experiment. By combining preference and performance measures both at the plant interspecific and intraspecific scales, we aimed to gain novel insights about the applicability of the PPH to phytophagous insects with an above-belowground life cycle and a clear assessment of the scale where it applies.

Materials and methods

Insects and plants

Eggs and females of *D. radicum* originated from a laboratory rearing initially constituted from a field population collected in the field in 2019 (Pleumeur-Gautier, Brittany, France, 48°48′11″N, 3°09′21″W). Flies were reared on rutabagas as described in Neveu Bernard-Griffiths (1998). Adults were identified based on sexual dimorphism in the size of the eye-spacing, which is much more pronounced in females than in males (Smith 1927). Females used in the experiments were 7–10 days old and mated, and all experiments took place at 20 ± 2 °C, 16L:8D, and 60 ± 10% RH. Four cultivars of *S. alba* were tested ('Brillant', 'Cezara', 'Sarah' and 'Verte'), as well as four cultivars of *B. rapa* ('Richi', 'Storkin', 'Tabaluga' and 'Yuki') and a reference cultivar of *B. oleracea* ('Parthenon'), all of which being commercial cultivars. Single seeds were sown in individual 3.5 × 3.5 × 5 cm clumps filled with potting soil (Premier Tech Horticulture) and plants were grown in a climatic chamber at optimum conditions (20 ± 1 °C, 16L:8D and 70 ± 10% RH) where they were watered twice a week. One week before the experiments started, plants were repotted individually in 7 × 7 × 6.5 cm plastic pots containing the same substrate. All plants were used at the 3–4 true leaves stage.

Table 1 Studies that have tested the PPH explicitly on phytophagous insects with aboveground adults and belowground larvae

Insect species	Plant observation scale	Treatments	PPH support	References
Delia radicum	Intraspecific	Two chemotypes	Yes	Van Leur et al. (2008)
Otiorhynchus sulcatus	Intraspecific	Two cultivars	No	Clark et al. (2012)
Otiorhynchus sulcatus	Intraspecific	Same plants attacked by conspecifics or not	No	Clark et al. (2011)
Sitona lepidus	Intraspecific	Same plants with nodules or not	Yes	Johnson et al. (2006)

Preference experiments

Female preference was investigated using a multi-choice set-up composed of a 50 cm cubic nylon cage (BugDorm, Taichung, Taiwan), which contained 10 plants randomly arranged in a circle and a water dispenser made from cotton at its center. When plants were repotted one week before the experiment, a 2 cm layer of clean river sand was added on top of the substrate to allow oviposition of the females and easy recovering of the eggs. The sand layer was separated from the potting soil by a 13 × 13 cm flexible plastic square that facilitated egg sampling. Two experiments were conducted to evaluate preference: an intraspecific test among either *S. alba* or *B. rapa* cultivars, and an interspecific test between the two species. In the intraspecific tests, two plants from each cultivar were placed in each cage, as well as two *B. oleracea* plants as reference. In the interspecific test, two cultivars of each species that were significantly different in the intraspecific test were used (two plants per cultivar per cage), to which *B. oleracea* was added as reference (two plants per cage). In all experiments, 20 females were released into the cage to lay eggs. After 24 h, the total number of eggs per plant was counted by separating these eggs from the sand by flotation. Twenty cages were set-up per experiment.

Performance experiment

To determine larval performance, a development experiment was performed on each of the nine cultivars used in the preference experiments. Ten fresh eggs randomly chosen from the rearing were deposited with a brush at the collar of an individual plant. Then, a plastic bag was fixed on top of the plant to allow recovering of the emerging adult flies at the end of their development. Thirty replicates per cultivar were performed. Two kinds of traits were assessed to characterize the larval performance. First, larval survival was estimated, which is the trait that most often correlates with female preference (Gripenberg et al. 2010). Larval survival was expressed as the emergence rate per plant, *i.e.* the proportion of eggs successfully developing into adults. Second, three life-history traits (LHT) of emerging adults were assessed: development time, hind-tibia length, and survival time with only water available. Hind-tibia length is known to be determined by host plant quality (Awmack and Leather 2002), and since survival time of unfed emerging adults is only determined by reserves and toxins accumulated by the larvae during their development, it is also an indirect estimator of larval performance. The sex of the emerging adults was also noted but, although LHT varied between females and males, no difference in sex ratio was observed between plant cultivars and the sex was not considered in subsequent analyses (Table S1).

Statistical analyses

All analyses were performed using the R software version 3.5.0 (R Core Team 2018). In all preference experiments, the proportions of eggs laid per cultivar were compared using Wald tests applied on multinomial models (R package 'RVAideMemoire'; Hervé 2020). The number of eggs laid on the two plants from the same cultivar was summed in the models. In interspecific preference tests, two cages were excluded from the analysis since no eggs were laid. Emergence rate was compared among cultivars using a likelihood ratio test applied on a Generalized Linear Model (GLM, family: quasibinomial, link: logit). Here and hereafter, pairwise comparisons of Estimated Marginal Means (EMMeans) were then performed (R package 'emmeans'; Lenth 2020) with *P*-values adjusted using the FDR correction (Benjamini and Hochberg 1995). In order to get an overall LHT-based index of larval performance to be compared between plant species and cultivars, a Principal Component Analysis (PCA) was performed on development time, hind-tibia length, and survival time (R package 'ade4'; Dray and Dufour 2007). Variables in this PCA were mean values per plant, which were centered and scaled prior to the analysis. Pearson's correlation tests were performed between original variables, and between original variables and PCA components. The first and second PCA components were retained as synthetic performance variables. These were compared between plant species and cultivars using nested ANOVAs. Pearson's correlation tests were also used to test the PPH, i.e. to assess the relationships between female preference and emergence rate, and between female preference and LHT-based indexes of larval performance. Correlation tests were only performed at the plant intraspecific scale, using mean values per cultivar. The interpretation was only graphical at the interspecific scale since each plant species was reduced to one point representing the mean value of the four cultivars (for *B. rapa* and *S. alba*).

Results

D. radicum females have both intra- and interspecific preferences

Intraspecific preference tests—When given the choice between four different cultivars of either *B. rapa* or *S. alba*, females showed significant intraspecific preferences. In *B. rapa*, cv. Richi received four times more eggs than the least preferred cultivars Tabaluga and Storkin (Fig. 1a). In *S. alba*, the least preferred cv. Verte received two times fewer eggs than the most preferred cultivars Sarah and Cezara (Fig. 1b). Moreover, more eggs were found on *B. rapa* and *S. alba* than on *B. oleracea* (Fig. 1).

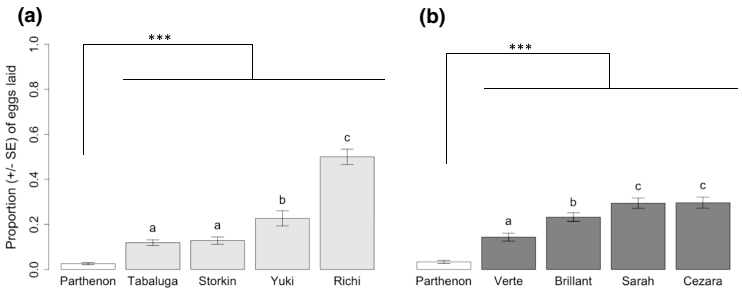


Fig. 1 Mean proportion (±SE) of eggs laid per cultivar by *Delia radicum* females, in **a** *Brassica rapa* and **b** *Sinapis alba*. Light grey: *B. rapa* cultivars, dark grey: *S. alba* cultivars, white: *Brassica oleracea* reference cultivar. ***: $P < 0.001$. Different letters indicate significant differences between cultivars of the same species. $N = 20$ cages per tested species

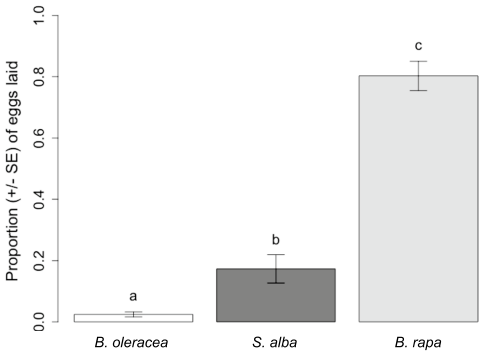


Fig. 2 Mean proportion (±SE) of eggs laid by *Delia radicum* females per plant species (white: *Brassica oleracea* reference cultivar, dark grey: *Sinapis alba* cultivars, light grey: *Brassica rapa* cultivars). Different letters indicate significant differences between species. $N = 18$ cages

Interspecific preference test – A significant preference was also observed when females were given the choice between two different cultivars of *B. rapa*, two cultivars of *S. alba* and the reference *B. oleracea.* At the interspecific scale, all three species were different from each other with *B. rapa* being the most preferred and *B. oleracea* the least preferred (Fig. 2). At the intraspecific scale, contrasts were the same as those observed in intraspecific preference tests (Fig. S1).

Larval survival differs between but not always within species

Emergence rate, here used as an estimate of larval survival, differed significantly between the three species ($F_{2,251} = 43{,}837$, $P < 0.001$; Fig. 3). At the interspecific scale, the emergence rate on *B. rapa* (mean ± SE 0.35 ± 0.02) was two to three times higher than on *B. oleracea* (0.20 ± 0.04) and *S. alba* (0.12 ± 0.01). Intraspecific differences were also observed but only among *S. alba* cultivars (Fig. 3).

Larval performance as estimated by life-history traits has two dimensions and differs between but not always within species

The PCA showed that the LHT-based estimate of larval performance can be captured by two, but not one, dimensions (Fig. 4). The first PCA component, hereafter named performance#1 variable, explained 59% of the variance and was mainly correlated with survival time and tibia length

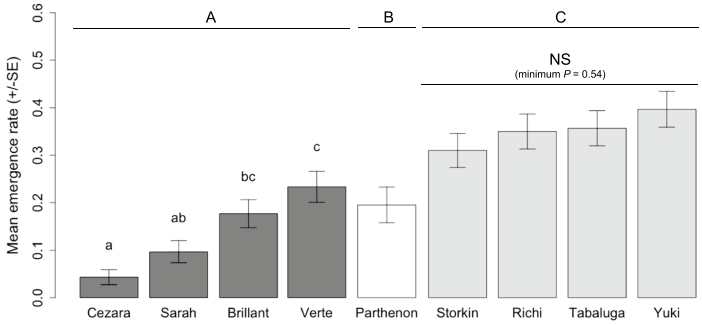


Fig. 3 Mean emergence rate (±SE) per plant of *Delia radicum*, depending on the plant species and the cultivar (dark grey: *Sinapis alba* cultivars, white: *Brassica oleracea* cultivar, light grey: *Brassica rapa* cultivars). Different uppercase letters indicate significant differences between species, whereas different lowercase letters indicate significant differences between *S. alba* cultivars. $N = 30$ plants per cultivar

(Fig. 4a and Table S2). The second component, hereafter named performance#2 variable, explained 33% of the variance and was mainly correlated with development time (Fig. 4a and Table S2). Correlations between LHT revealed by the PCA were not biased by differences between plant species or cultivars, since similar trends were observed within cultivars (Table S3). Larval performance#1 did not differ between species ($F_{2,185} = 0.394$, $P = 0.675$) and within *S. alba* but differed significantly between *B. rapa* cultivars. Indeed, cv. Richi and Yuki were more favorable to larvae than cv. Tabaluga and Storkin (Fig. 4b). On the contrary, larval performance#2 significantly differed between species ($F_{2,185} = 9.779$, $P < 0.001$), with *S. alba* being less favorable than the two other species, but not within species ($F_{6,185} = 1.511$, $P = 0.177$) (Fig. 4c).

The Preference–Performance Hypothesis is partially supported and only in B. rapa

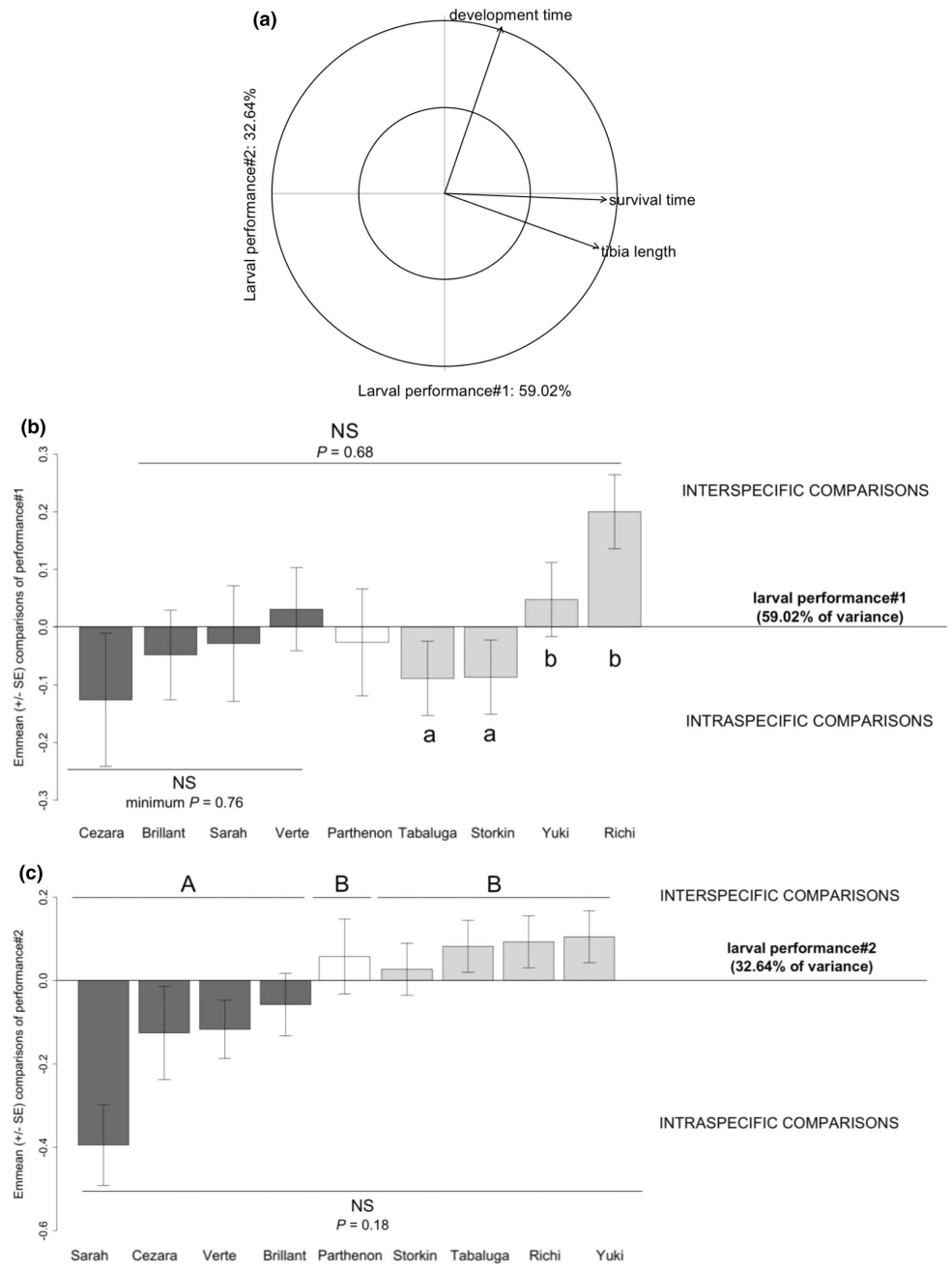
No correlation was found between female preference and larval performance#2 or emergence rate in *B. rapa* (respectively $r = 0.476$, $df = 2$, $P = 0.524$; $r = 0.157$, $df = 2$, $P = 0.843$) (Fig. S2A and S2B). However, a strong positive correlation was found with larval performance#1 ($r = 0.978$, $df = 2$, $P = 0.022$; Fig. 5a), supporting the PPH in this species. In *S. alba*, no positive correlation was found with any LHT-based performance variable (performance#1: $r = -0.788$, $df = 2$, $P = 0.212$; performance#2: $r = -0.482$, $df = 2$, $P = 0.518$) (Fig. S2C and S2D) as well as with emergence rate, refuting the PPH in this species. The correlation was even almost significantly negative with the emergence rate in this species ($r = -0.941$, $df = 2$, $P = 0.059$, Fig. 5b). At the interspecific scale, the graphical interpretation did not allow the observation of any clear correlation trend between female preference and the different larval performance variables. However, partial support for the PPH was found. Indeed, although the least preferred host species for oviposition (*B. oleracea*) was not the one conferring the worst larval development, the host species largely preferred by females (*B. rapa*) was the one allowing the best larval development (Fig. S3).

Discussion

The relationship between female oviposition preference and offspring performance is a central question in the study of host plant selection by phytophagous insects. One of the main hypotheses that have been proposed to explain this relationship is the Preference–Performance Hypothesis (PPH, Jaenike 1978) which states that female oviposition preference should be positively correlated with offspring performance. In our study, this correlation was assessed both between three host plant species and within two of these species. No correlation was found neither at the plant interspecific scale nor at the intraspecific scale within *S. alba,* but a positive relationship was found within *B. rapa*. In congruence with our results, previous studies conducted on *D. radicum* have already shown contrasts in female preference both between brassicaceous species (Kergunteuil et al. 2015a; Jyoti et al. 2001; Lamy et al. 2017) and within *B. rapa* (Lamy et al. 2020), as well as differences in larval performance both between and within several brassicaceous species (Dosdall et al. 1994, 2000; Shuhang et al. 2016; Santolamazza-Carbone et al. 2017). Our work adds novel insights to the understanding of the oviposition behavior of females of phytophagous insects with an above-belowground life cycle.

As already pointed out by Gripenberg et al. (2010), our results show that the variable used to estimate larval performance has a great influence on the conclusions regarding the support of the PPH. Indeed, conclusions may differ depending on the variable used. Here, performance was defined as larval survival and life-history traits of emerging adults, that latter not being reducible to a single variable but having two independent components (each characterized by one or two traits). Depending on the performance variable used, a relationship between female preference and larval performance was found significant or not. These results are a reminder that multiple variables estimating larval performance are needed to draw general conclusions about the PPH.

This study also demonstrates that support for the PPH may sometimes be found at only one plant observational scale, here the intraspecific scale. This highlights that the determinants of host plant selection may differ between and within plant species (Bernays and Chapman 1994). As argued by Jones et al. (2019), these two scales should at least be differentiated in global analyses. We further suggest that future studies aimed at testing the validity of the PPH should consider both observation scales to be fully relevant. Moreover, our results show that at the intraspecific scale, the PPH can be supported in some plant species but not in others. Beyond highlighting that the host selection strategy can differ within different species of the host range, this demonstrates that behaving as predicted by the PPH is not an intrinsic characteristic of the insect species, but results from an interactive effect of the insect species and the plant


(a)
development time
survival time
tibia length
Larval performance#2: 32.64%
Larval performance#1: 59.02%
(b)
NS
P = 0.68
INTERSPECIFIC COMPARISONS
larval performance#1
(59.02% of variance)
b
b
a
a
INTRASPECIFIC COMPARISONS
NS
minimum P = 0.76
Emmean (+/- SE) comparisons of performance#1
Cezara
Brillant
Sarah
Verte
Parthenon
Tabaluga
Storkin
Yuki
Richi
(c)
A
B
B
INTERSPECIFIC COMPARISONS
larval performance#2
(32.64% of variance)
INTRASPECIFIC COMPARISONS
NS
P = 0.18
Emmean (+/- SE) comparisons of performance#2
Sarah
Cezara
Verte
Brillant
Parthenon
Storkin
Tabaluga
Richi
Yuki

◂**Fig. 4** **a** Correlation circle of the Principal Component Analysis performed on the three life-history traits estimating the larval performance of *Delia radicum*. **b** EMMeans comparisons of larval performance#1 (=PCA component 1) between species (above bars) and within species (below bars); **c** Same as **b** for larval performance#2 (=PCA component 2). Dark grey: *Sinapis alba* cultivars, white: *Brassica oleracea* cultivar, light grey: *Brassica rapa* cultivars. Different letters indicate significantly different species or cultivars

species. Finally, the use of nine cultivars of three different species allowed us to assess the validity of the PPH quantitatively. Although this quantitative dimension adds some variance and can provide ambiguous results, such as the partial support (or the partial refutation) we found at the plant interspecific scale, we believe that it increases the level of relevancy of PPH assessments compared to qualitative tests involving two treatments only. We thus encourage more quantitative analyses to be performed.

Johnson et al. (2006) suggested including insects with both above- and belowground life stages in the preference–performance debate. Very few studies followed this suggestion. Our results agree with Johnson et al. (2006) about the relevancy of testing the PPH in such insect species. The PPH was supported in one plant species, *B. rapa*, which is in line with Van Leur et al. (2008) who found the same result when measuring preference and performance of *D. radicum* on two chemotypes of another plant species. However, our results refute the PPH in a second plant species, *S. alba*. This demonstrates the non-universality of the PPH and the need for more studies in above-belowground insects to get a global picture of its application to these species. Something striking about this study is that there is an almost significant negative correlation between female preference and emergence rate in *S. alba* as females preferentially laid eggs on cultivars providing the lowest larval survival. Many other studies have failed to find a positive correlation between female preference and offspring performance in other species, and several hypotheses have been suggested to explain this seemingly bad motherhood behavior (Mayhew 1997, 2001). The enemy-free space is one of them, stating that host plant selection is partly based on a trade-off between plant quality and natural enemy avoidance. Females may indeed lay eggs preferentially on lower quality hosts if the risk of larvae being attack by natural enemies is reduced (Ballabeni et al. 2001; Moon and Stiling 2006). Another hypothesis is that female preference may be correlated with its own performance rather than larval performance (Scheirs et al. 2000, 2004; Scheirs and Bruyn 2002). It is also possible that host plant cues, especially chemical ones, are misinterpreted by the females. Indeed, chemical cues used by *D. radicum* females for oviposition may not correlate with determinants of the plant's quality, or with chemical cues used by larvae for root acceptance. This may even be likely in cultivated species such as the ones we used that have been artificially selected for nutritional traits. Whether the behavior we observed in *D. radicum* females is adaptive in some way or is a consequence of uncoupling of chemical signals used by females and determinants of larval performance during the domestication process in *S. alba* remains to be tested.

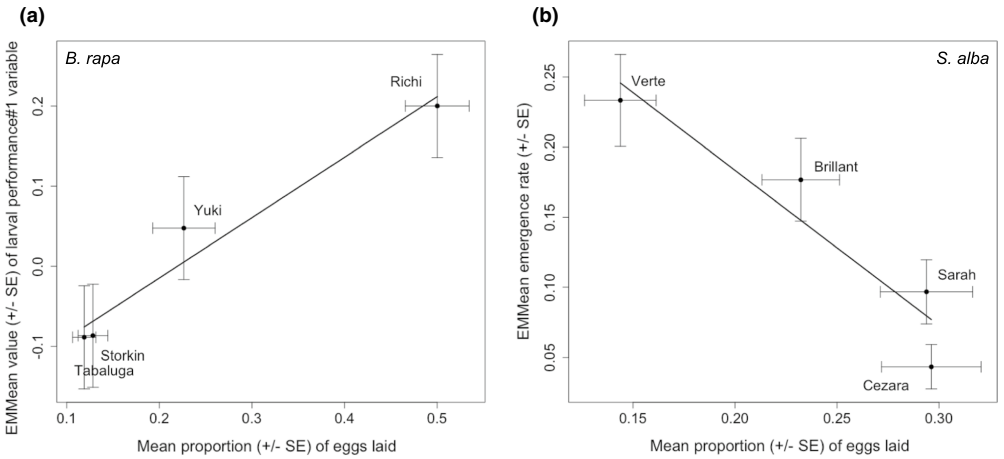


Fig. 5 **a** Relationship between the female preference of *Delia radicum* and larval performance#1 in *Brassica rapa* (cv: 'Tabaluga', 'Storkin', 'Yuki' and 'Richi') (r=0.978, df=2, P=0.022). **b** Relationship between female preference and emergence rate in *Sinapis alba* (cv: 'Verte', 'Brillant', 'Sarah' and 'Cezara') (r= − 0.941, df=2, P=0.059)

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Author contribution statement AMC, MH, and KM conceived and designed the experiments. KM performed the experiments and collected the data. MH and KM analyzed and interpreted the data. KM wrote the first draft and all authors provided editorial advice.

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Availability of data and material The datasets generated during and/or analysed during the current study are available in the Figshare repository, https://doi.org/10.6084/m9.figshare.14994120.v3

Code availability Not applicable.

Declarations

Conflict of interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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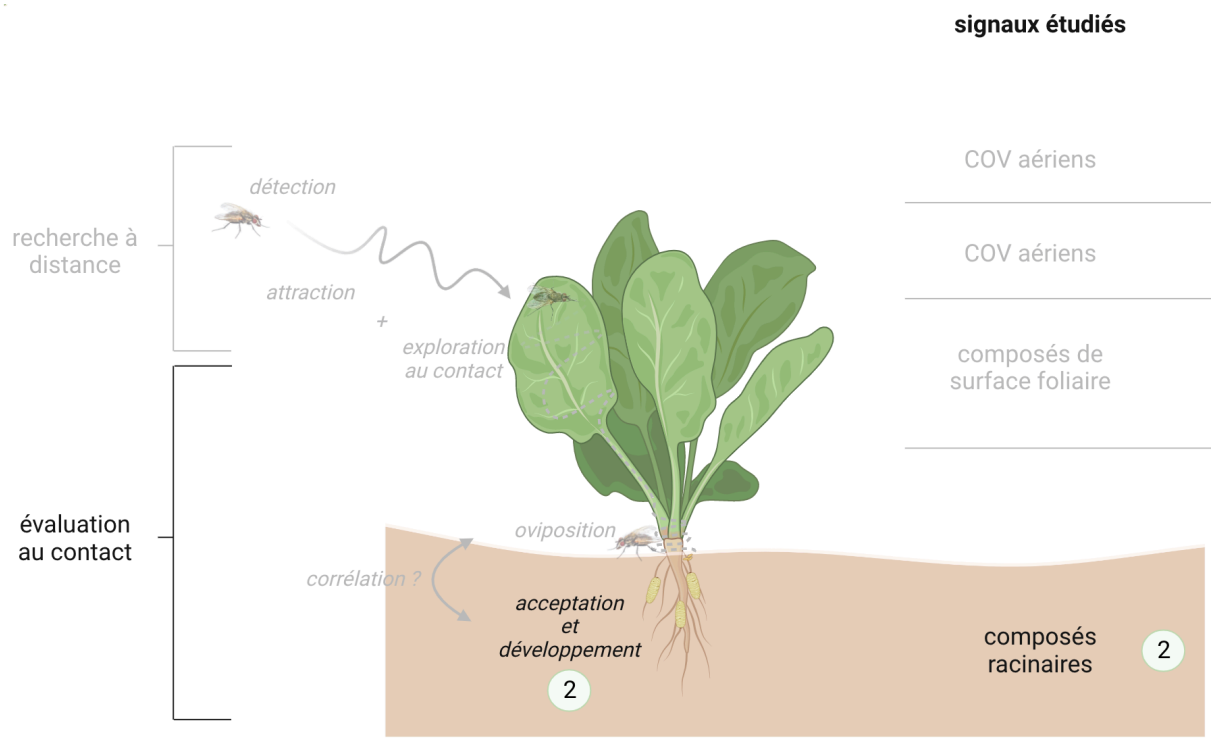
Article 2

Root compounds mediate host acceptance in larvae of an above-belowground specialist phytophagous insect



Root compounds mediate host acceptance in larvae of an above-belowground specialist phytophagous insect

Manuscript in preparation

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Anne Marie Cortesero and Maxime Hervé equal contribution last author

ABSTRACT

Host plant selection in phytophagous insects is largely governed by females, which choose the where their offspring will develop. Despite their limited mobility and subsequent dependence on females' choices, insect larvae that live belowground are able to perceive root compounds and to use these to decide whether the plant should be accepted as a host or rejected. A recent study on a specialist of Brassicaceae, the cabbage root fly (*Delia radicum*), showed contrasts in larval development between three host species, namely *Brassica rapa*, *B. oleracea* and *Sinapis alba*, as well as within *B. rapa* and *S. alba*. In the present study, we aimed to assess whether these contrasts originate from differences in host acceptance by larvae, and to progress into the identification of root compounds that would mediate such effect. For this purpose, a combination of whole plant experiments and feeding tests on artificial substrates supplemented with root extracts were performed, using a bioguided fractionation approach applied on the three plant species as well as on two cultivars of *B. rapa* et *S. alba*. Our results show that larval feeding is stimulated by a synergy between polar and less polar root compounds in *B. rapa* and *B. oleracea*, and that polar compounds mediate deterrence or non-acceptance in *S. alba*. This study complements the scarce literature on host acceptance mechanisms in belowground insect larvae, and highlights the importance of integrating multiple plant species when studying host recognition cues, as different ones can mediate host acceptance in different species and populations of a given insect's host range.

Keywords: belowground specialist phytophagous insects; root compounds; larval host acceptance; plant-insect interactions

INTRODUCTION

In phytophagous insects, host plant selection is mainly achieved by adult females, which choose the site where their offspring will develop. Indeed, in many species larval stages have low dispersal abilities and energy reserves (Thompson, 1988; Jaenike, 1990). Therefore, the quantity as well as the quality of food available for larval development is a direct result of maternal choices (Gripenberg *et al.*, 2010). When host plants differ in their quality, the 'mother knows best' hypothesis predicts a positive relationship between female preferences for oviposition sites and offspring performance (Jaenike 1978; Valladares and Lawton 1991). Although this hypothesis has been verified in many species (e.g. Keeler and Chew 2008; Zhang *et al.*, 2012; Du *et al.*, 2016), it is not systematic (e.g. Mayhew 1997, 2001; Scheirs *et al.*, 2000; Jones *et al.*, 2019, Menacer *et al.*, 2021). In case of no relationship, usual drivers of host plant choices are the own female's survival (Scheirs *et al.*, 2000, 2004; Scheirs and Bruyn 2002) or the predation risk for larvae (Ballabeni *et al.*, 2001; Moon and Stiling 2006). However, another possible explanation is that although the oviposition site is chosen by adult females, larvae can also take the decision to accept or reject the plant on which they were deposited, even if their mobility is limited (Soler *et al.*, 2012; Rojas *et al.*, 2018).

Host discrimination abilities have been extensively studied in aboveground larvae (*e.g.* del Campo *et al.*, 2001; Schoonhoven *et al.*, 2005; Miles *et al.*, 2005). However, many phytophagous insects such as some Diptera, Lepidoptera, Coleoptera and Orthoptera, have a belowground larval stage in their life cycle (Brown and Gange, 1990). These larvae usually have lower dispersal abilities than aboveground ones, making them even more dependent on the mother's choice and presumably less able to discriminate plant species. However, according to Johnson *et al.* (2006) similarities exist between above- and belowground larvae, especially regarding contact chemoreception, suggesting that in belowground environments larvae could use plant cues to identify their hosts. Indeed, belowground larvae use chemical compounds released by roots as foraging cues and once at contact, they can assess the quality of the plant by biting into the roots and directly tasting surface and internal compounds; these 'test bites' finally determine whether the plant is accepted or not (Johnson and Gregory, 2006; van Dam, 2009). Since roots of different plant species differ in both their primary and specialized metabolites, root herbivores can be expected to use these differences to discriminate plants (Erb *et al.*, 2013). However, in addition to being scarce, studies on the effect of internal and surface root metabolites focus extensively on a single plant species, not allowing concluding on whether the chemical cues used by larvae are specific to this species or whether they are a more general cue for its entire host range. How much such cues influence larval food choice belowground and how specific these are remains largely unknown.

Adults of the cabbage root fly, *Delia radicum* (Diptera: Anthomyiidae), live aboveground and females lay their eggs near the stem of brassicaceous plants (Capinera, 2008). Once hatched, larvae move into the soil where they feed on plant roots. Newly hatched larvae have been shown to respond to olfactory

cues emitted by brassicaceous plants (Košťál, 1992), and to use species-specific root exudates to locate suitable hosts (Ross and Anderson, 1992; Johnson and Gregory, 2006). Furthermore, they possess chemoreceptors on their cephalic lobes that are thought to have gustatory functions (Ryan & Behan, 1973), which would allow them to perceive surface and internal plant compounds. A recent study showed that the development success of larvae varies with the plant species, with a more successful development on Chinese cabbage (*B. rapa* subsp. *pekinensis*) and cabbage (*Brassica oleracea* L.) than on the white mustard (*Sinapis alba* L.) (Menacer *et al.*, 2021). Moreover, differences were also shown at the intraspecific scale, namely between *B. rapa* and *S. alba* populations (Menacer *et al.*, 2021). Although root compounds such as nutrients and toxins may explain these contrasts, it can be hypothesized that they originate, at least partly, from differences in host acceptance by larvae. The present study aimed to test this hypothesis using a combination of whole plant experiments and feeding tests on artificial substrates supplemented with root extracts. A bioguided fractionation approach was followed to provide further insights into the identification of root compounds involved in the behaviors observed.

MATERIALS AND METHODS

Insects and plants

Eggs and larvae of *D. radicum* originated from a laboratory rearing initially constituted from a field population collected in the field in 2019 (Pleumeur-Gautier, Brittany, France, 48°48′11″N, 3°09′21″W). Flies were reared on rutabaga in controlled conditions as described in Neveu Bernard-Griffiths (1998). All experiments were conducted with first instar larvae aged one day after hatching. Experiments took place in a climatic controlled room at 20 ± 2 °C, 16L:8D, and 60 ± 10 % RH. Two cultivars of *S. alba* were tested (‘Sarah’ and ‘Verte’) as well as two cultivars of *B. rapa* subsp. *pekinensis* (‘Richi’ and ‘Tabaluga’) and a reference cultivar of *B. oleracea* (‘Parthenon’). All cultivars tested have been shown to be good host plants for *D. radicum* larvae, except the resistant *S. alba* cultivar Sarah, on which larval development is poor (Menacer *et al.*, 2021). Single seeds were sown in individual 3.5 × 3.5 × 5 cm clumps filled with a fertilized substrate (Premier Tech Horticulture), and plants were grown in a climatic chamber at optimal conditions (20 ± 1 °C, 16L:8D and 70 ± 10% RH) where they were watered twice a week with a nutritive solution (Hoagland and Arnon, 1950). One week before experiments started, plants were repotted individually in 7 × 7 × 6.5 cm plastic pots containing the same substrate as for sowing. All plants were used at the 4–5 true leaves stage.

Larval root consumption

To estimate root consumption by larvae, the total length of the root system of infested plants was compared to the one of uninfested plants from the same cultivar. Plants were infested by depositing ten

fresh eggs, randomly chosen from the rearing, at the collar of each individual plant. Twenty-four days later, plants were removed from the substrate, their roots were cut and carefully washed. Roots were then gently dried on absorbent paper and pictured under standardized light conditions and distance. The total length of the root system was then measured using ImageJ® and RootGraph®. Fourteen to fifteen replicates were performed per treatment and cultivar.

Extraction and fractionation of root chemical compounds

Plants were carefully removed from the substrate and their roots gently washed. After cleaning, roots were cut, quickly dried on absorbent paper and weighed, before they were placed into a 15 ml Falcon tube and frozen into liquid nitrogen to stop metabolic activity. They were then lyophilized for 72 h and ground into fine powder for 20 seconds at 3500 rpm using a BeadBug™ D1030 (E) with 2.8 mm stainless beads. Ten samples of 1-3 plants each were constituted per cultivar, representing 450 mg fresh weight (FW) per sample. Fractionation was realized by using a sequence of three extraction solvents of increasing polarity: hexane (Hex) for apolar compounds, then dichloromethane (DCM) for moderately polar compounds, then methanol (MeOH) for polar compounds. For each cultivar, each of the 10 samples was put into a glass beaker to which 5 ml of solvent was added. Samples were then agitated for 1h15 at 200 rpm, after which supernatants were collected and transferred into 10 ml glass tubes. The same process was repeated with the following solvents, leading to three fractions per sample. Solvents were then completely evaporated under nitrogen flow and samples resuspended in 1.5 ml of the original solvent.

Following results of the feeding tests (see below), active fractions were sub-fractionated. Sub-fractionation was realized by Solid Phase Extraction (SPE) using CHROMABond ® cartridges filled with 500 mg of a C8 stationary phase for the methanol fraction or of a SiOH stationary phase for dichloromethane and hexane fractions. For each of the same samples used above, three sub-fractions were obtained by loading 200 µl of sample on a cartridge, followed by elution with 4 ml of three solvents of different polarity (respectively and in this order: H_2O, H_2O:MeOH 50:50 and MeOH for the methanol fraction; DCM:MeOH 98:2, DCM:MeOH 90:10 and MeOH for the dichloromethane fraction; Hex:DCM 85:15, DCM and DCM:MeOH 50:50 for the hexane fraction). Sub-fractions were collected in glass tubes, completely evaporated under nitrogen flow and resuspended in 1.5 ml of the same solvent.

Feeding tests on agar disks

The feeding test consisted in dual choices between two agar disks (agar 3 %, diameter 5 mm, height 2 mm) placed in a closed plastic Petri dish (diameter 26 mm) where eight first-instar larvae were deposited at the center. On each agar disk, 7 µl of root extract (or control solution) were deposited as to cover the upper face, lower face and edges of the disk. The solvent was then let to evaporate entirely before the

test started. The same root extract was used for all disks of the same treatment, which consisted in a pool of the 10 samples obtained above and mixed in equal proportions. Root extracts were prepared at natural concentrations, meaning that they contained compounds extracted from a leaf biomass equivalent to that of the agar biomass impregnated with 7 µl of solvent (i.e. 18 mg FW per disk, determined in preliminary experiments with colored solutions). Once larvae were placed in the dish, the number of larvae feeding on each disk was counted every 5 min during 45 min. Observations were made with a numeric binocular microscope (Adonstar™ ADSM302). A larva was considered to be feeding when it was active in, on or under the disk, or on its sides.

In a first series of experiments, total root extracts were tested. These were reconstituted by deposing 7 µl of each of the three fractions on the agar disks. Then, each fraction was tested alone. Tests of pairs of fractions were realized when the fractions alone did not reproduce the effect observed with total extracts. For the fraction (or combination of fractions) that reproduced this effect, sub-fractions were subsequently tested alone or in combination. Depending on the experiment, root extracts were compared either to a control solution (same pure solvent as present in the root extract) or to root extracts of the second cultivar of the same species. Twenty replicates were performed per experiment in the first case, 30 replicates in the second case. In the special case of root extracts of *S. alba*, for which phagodeterrent effects were suspected, sucrose was also included in the agar disks. Sucrose was chosen as it is known to be a major phagostimulant compound for larvae and insects in general (Hopkins et al., 1999; Brown and Gange, 1990; Chapman, 2003). Sucrose was included at a concentration of 20 mM, as preliminary experiments showed that it was the lowest concentration stimulating feeding of *D. radicum* larvae (Fig. S1).

Statistical analyses

All analyses were performed in the R software version 4.0.2 (RStudio Team, 2019) using the following packages: 'lme4' (Bates et al., 2015), 'emmeans' (Lenth, 2022), 'car' (Fox and Weisberg, 2019) and 'RVAideMemoire' (Hervé, 2022). For larval root consumption, the total length of the root system was compared between infested and uninfested plants separately for each cultivar using Welch *t* tests. For feeding tests, the number of feeding observations per disk was compared between the two treatments separately for each experiment, using Wald tests applied on Generalized Linear Mixed Models (distribution: negative binomial, link function: log). The treatment was used as a fixed factor and the replicate as a random factor. Two analyses were performed per experiment: one per observation time point to detect behavioral kinetics, and one on the summed feeding observations to assess global effects.

RESULTS

Larvae consume roots of all cultivars but the resistant S. alba

The total length of the root system differed significantly between the two treatments for *B. oleracea* and for the two cultivars of *B. rapa*, with shorter roots for infested plants than for uninfested ones (Parthenon: $t = -3.06$, df = 24.43, $p = 0.005$; Richi: $t = -4.62$, df = 23.54, $p < 0.001$; Tabaluga: $t = -2.53$, df = 13.99, $p = 0.024$). In *S. alba*, the same result was obtained with cultivar Verte whereas no difference was observed for cultivar Sarah (Verte: $t = -6.35$, df = 18.71, $p < 0.001$; Sarah: $t = -0.21$, df = 26.53, $p = 0.839$) (Fig. 1). Then, it is confirmed that *D. radicum* larvae consume roots of all favorable cultivars, suggesting that roots of these cultivars contain phagostimulant compounds. On the contrary, no consumption is detected on the resistant *S. alba* cultivar, suggesting either a lack of phagostimulant compounds or the presence of phagodeterrent compounds.

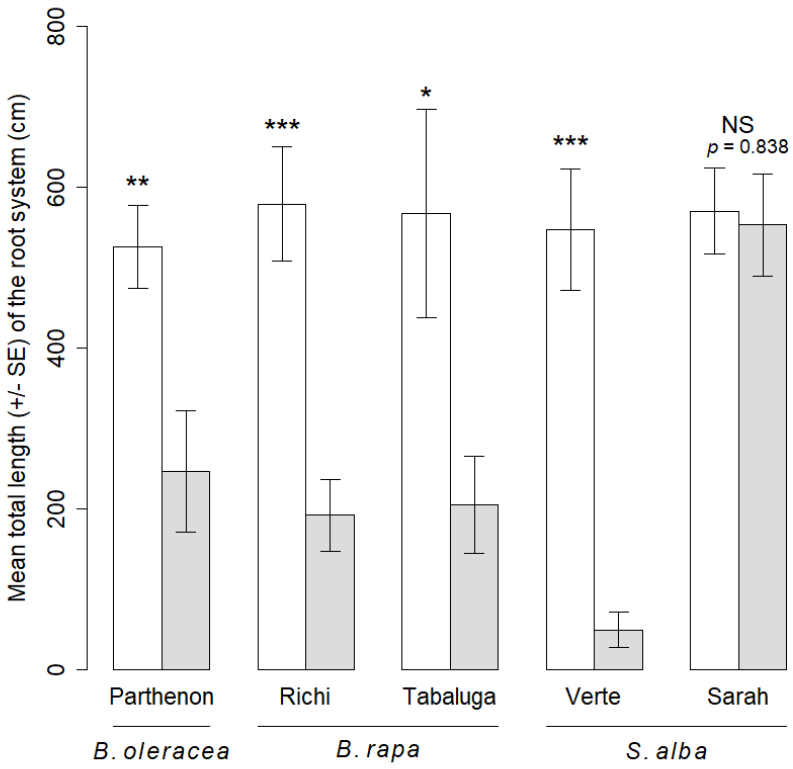

Fig. 1 Mean total length (+/- SE) of the root system of uninfested (white) and infested (grey) plants. N = 14-15 per treatment and cultivar. NS: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Roots of* B. oleracea *contain polar and non-polar phagostimulant compounds that act synergistically

The mean total number of feeding observations was significantly higher on disks with a total root extract of *B. oleracea* than on control disks ($\chi^2 = 21.85$, df = 1, $p < 0.001$) (Fig. 2a). Fractionation of the total extract was therefore performed to identify the fraction(s) responsible for this effect. The contrast in feeding intensity observed with the total root extract was not reproduced with any of the three fractions (hexane fraction: $\chi^2 = 0.32$, df = 1, $p = 0.571$; dichloromethane fraction: $\chi^2 = 1.00$, df = 1, $p = 0.316$; methanol fraction: $\chi^2 = 0.82$, df = 1, $p = 0.364$) (Fig. 2b). Therefore, the interactive effect of these fractions was tested by combining them by pairs. The contrast initially observed with the total extract was reproduced when combining the hexane and methanol fractions ($\chi^2 = 20.93$, df = 1, $p < 0.001$) (Fig. 2c). No significant difference was found when the hexane and dichloromethane ($\chi^2 = 0.13$, df = 1, $p = 0.722$) or dichloromethane and methanol ($\chi^2 = 1.74$, df = 1, $p = 0.188$) fractions were combined (Fig. 2c). Sub-fractionation of the hexane and methanol fractions and combinations were therefore performed. The feeding contrast observed previously was reproduced with the combination of the methanol most polar sub-fraction 1 and the hexane less polar sub-fraction 1 ($\chi^2 = 4.70$, df = 1, $p = 0.030$) (Fig. 3a), as well as with the combination of the methanol most polar sub-fraction 1 and the hexane most polar sub-fraction 3 ($\chi^2 = 5.04$, df = 1, $p = 0.025$) (Fig. 3c). On the contrary, a significant opposite contrast was observed when combining the methanol less polar sub-fraction 3 and the hexane less polar sub-fraction 1 ($\chi^2 = 17.43$, df = 1, $p < 0.001$) (Fig. 3a). No differences were observed for the other combinations (Fig 3) (Table S1).

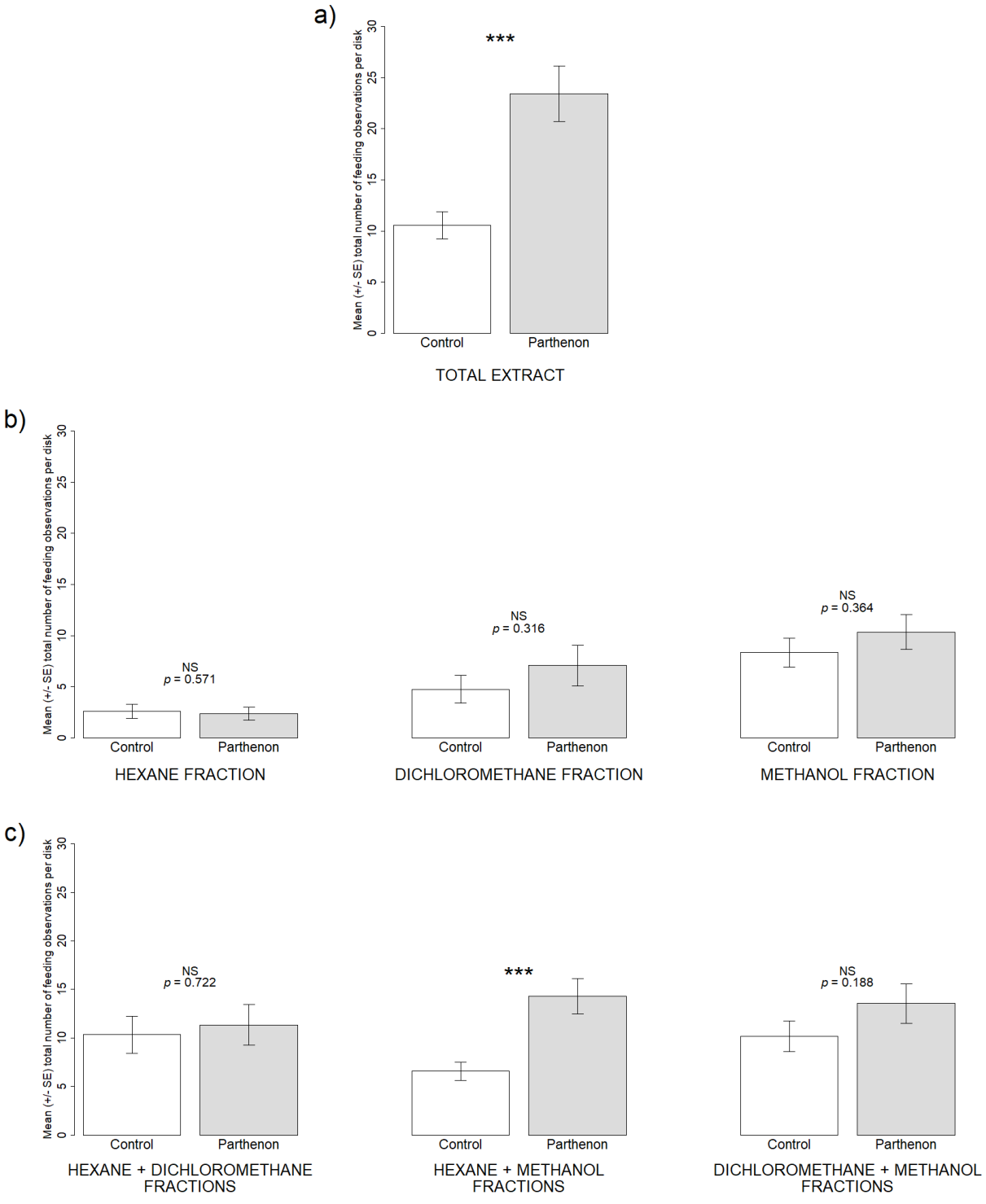

Fig. 2 Mean (± SE) total number of feeding observations per agar disk supplemented either with solvents only (Control) or (a) the total root extract of *B. oleracea* (cv. Parthenon), (b) one fraction of the total extract (c) two fractions of the total extract. NS: $p > 0.05$, ***: $p < 0.001$. N = 20 per experiment. See behavioral kinetics in Fig. S2.

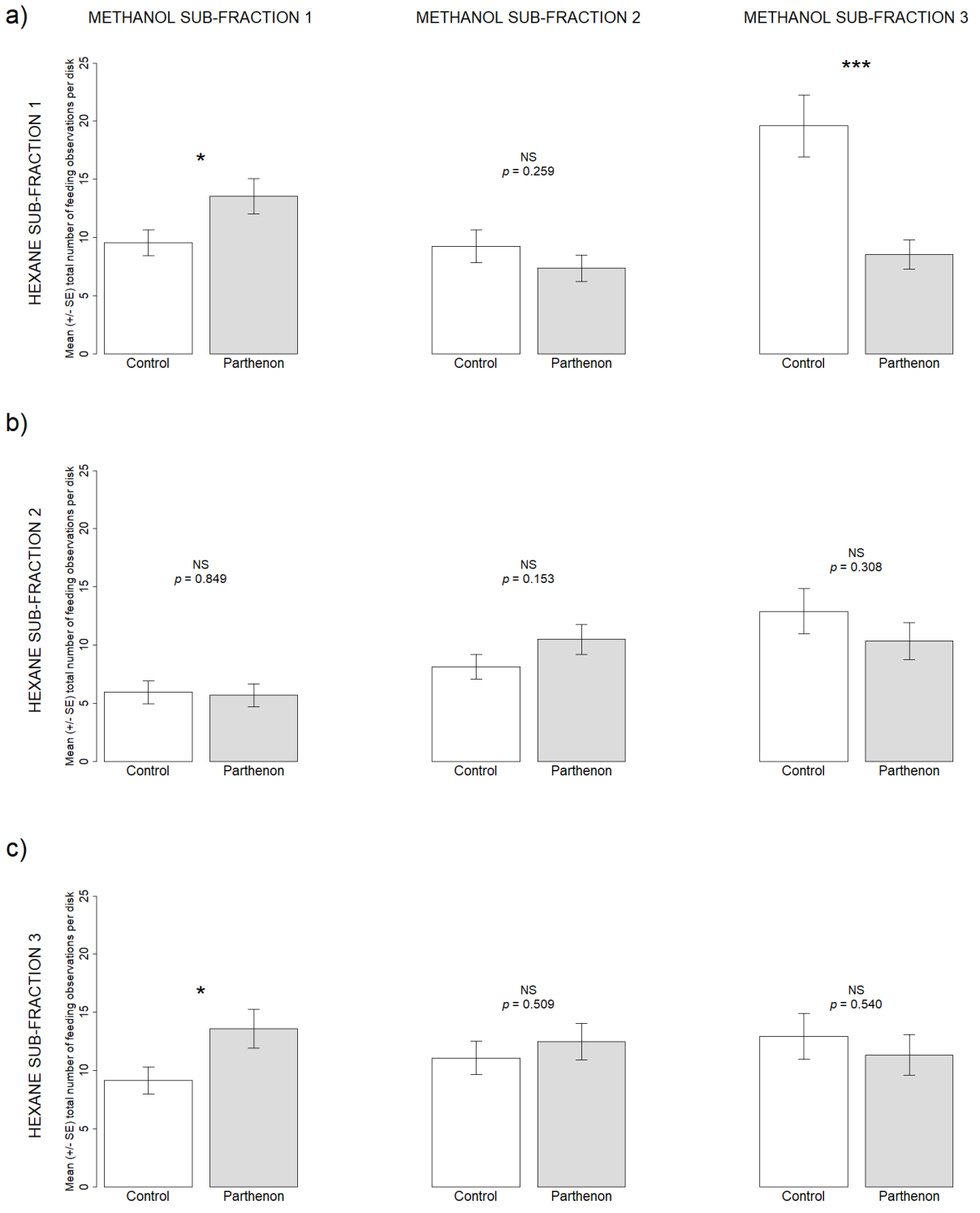

Fig. 3 Mean (± SE) total number of feeding observations per agar disk either supplemented with solvents only (Control) or (a) hexane sub-fraction 1 + one of each methanol sub-fraction, (b) hexane sub-fraction 2 + one of each methanol sub-fraction, (c) hexane sub-fraction 3 + one of each methanol sub-fraction of the root extract of *B. oleracea* (cv. Parthenon). NS: $p > 0.05$, *: $p < 0.05$, ***: $p < 0.001$. Behavioral kinetics in Fig. S3.

Roots of **B. rapa** *contain polar phagostimulant compounds that act with less polar compounds*

The mean total number of feeding observations was significantly higher on disks with a total root extract of *B. rapa* cv. Richi than on control disks ($\chi^2 = 5.81$, df = 1, $p = 0.016$) (Fig. 4a), whereas no difference was observed for cv. Tabaluga ($\chi^2 = 0.24$, df = 1, $p = 0.624$). When total extracts of the two cultivars were confronted to each other, no contrast was observed ($\chi^2 = 2.35$, df = 1, $p = 0.125$) (Fig. 4a). Since only cv. Richi showed a clear effect, it was chosen to perform further fractionation and identify the fraction(s) responsible for this effect. As in *B. oleracea*, the contrast in feeding intensity observed with the total extract was not reproduced with any of the three fractions (hexane fraction: $\chi^2 = 0.15$, df = 1, $p = 0.700$; dichloromethane fraction: $\chi^2 = 2.20$, df = 1, $p = 0.138$; methanol fraction: $\chi^2 = 1.07$, df = 1, $p = 0.302$) (Fig. 4b). Therefore, the interactive effect of these fractions was tested by combining them by pairs. The contrast initially observed with the total extract was reproduced when combining the hexane and methanol fractions ($\chi^2 = 4.78$, df = 1, $p = 0.029$), as well as the dichloromethane and methanol fractions ($\chi^2 = 16.62$, df = 1, $p < 0.001$) (Fig. 4c). No significant difference was found when the hexane and dichloromethane fractions were combined ($\chi^2 = 1.45$, df = 1, $p = 0.228$) (Fig. 4c).

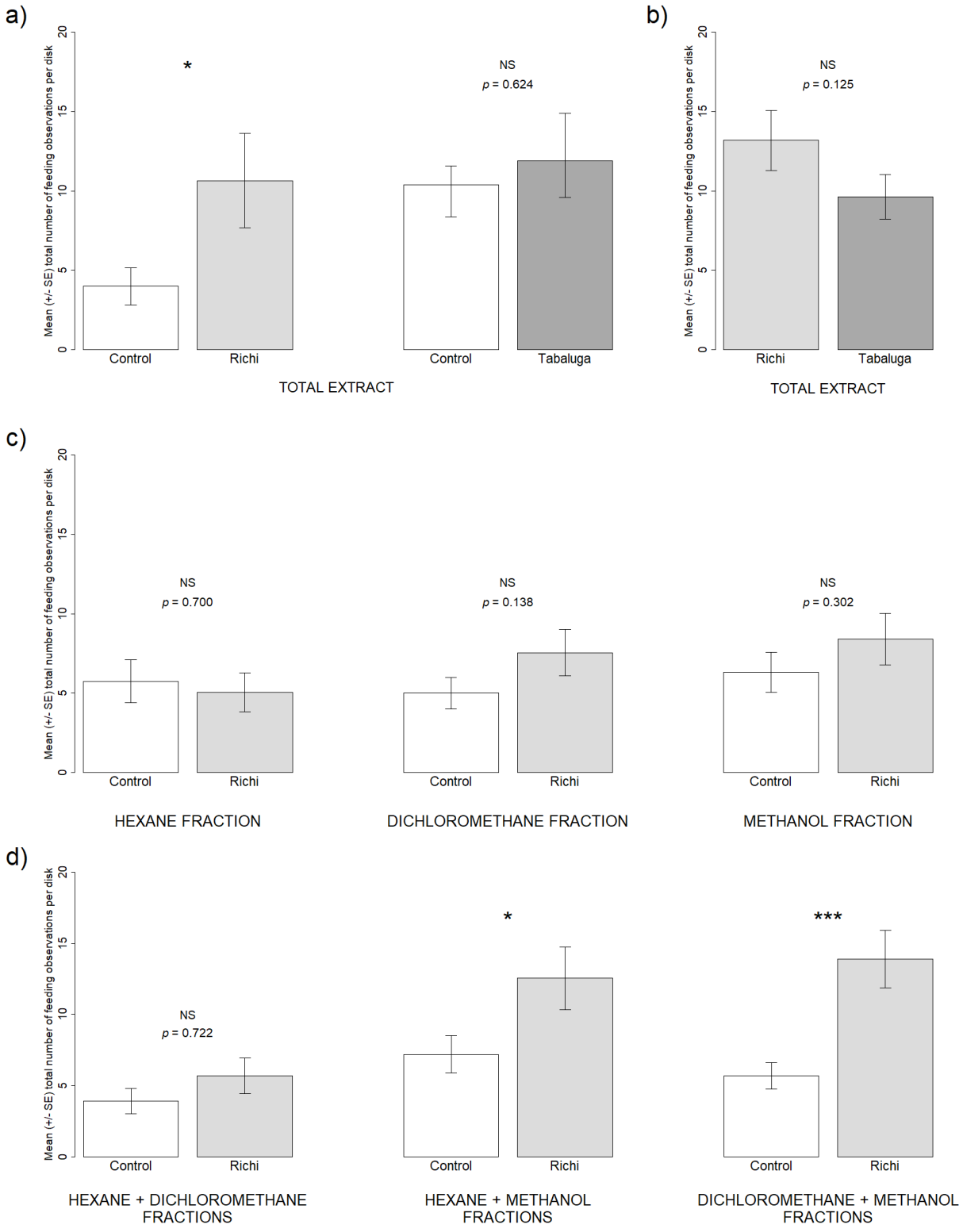

Fig. 4 Mean (± SE) total number of feeding observations per agar disk either supplemented with solvents only (Control) or (a) the total root extract of *B. rapa* (cv. Richi), (b) the total root extract of each cultivar, (c) one fraction of the total extract, (d) two fractions of the total extract. NS: $p > 0.05$, *: $p < 0.05$, ***: $p < 0.001$. N = 20 per experiment except for (a, right) where N = 30. See behavioral kinetics in Fig. S4.

***Highly polar compounds induce intraspecific contrasts in feeding intensity in* S. alba**

For the two cultivars tested, the mean total number of feeding observations did not differ between disks with a total root extract of *S. alba* and control disks (cv. Sarah: $\chi^2 = 0.04$, df = 1, $p = 0.846$; cv. Verte: $\chi^2 = 2.00$, df = 1, $p = 0.237$) (Fig. 5a). However, when total extracts of these cultivars were confronted to each other, the feeding intensity was significantly lower on disks to which a root extract of cv. Sarah was added ($\chi^2 = 10.56$, df = 1, $p = 0.001$) (Fig. 5a). Fractionation of total extracts was therefore performed to identify the fraction(s) responsible for this effect, and fractions tested by comparing the two cultivars. The contrast observed with total extracts was reproduced with the methanol fraction ($\chi^2 = 8.61$, df = 1, $p = 0.003$), whereas no difference was observed with the hexane ($\chi^2 = 0.16$, df = 1, $p = 0.691$) and dichloromethane fractions ($\chi^2 = 3.4979$, df = 1, $p = 0.061$) (Fig. 5b). Sub-fractionation of the methanol fraction was therefore performed. The contrast in feeding intensity observed with the methanol fraction was reproduced with the most polar sub-fraction 1 ($\chi^2 = 6.64$, df = 1, $p = 0.01$), whereas no difference was observed with the less polar sub-fractions 2 ($\chi^2 = 0.51$, df = 1, $p = 0.476$) and 3 ($\chi^2 = 0.12$, df = 1, $p = 0.728$) (Fig. 5c).

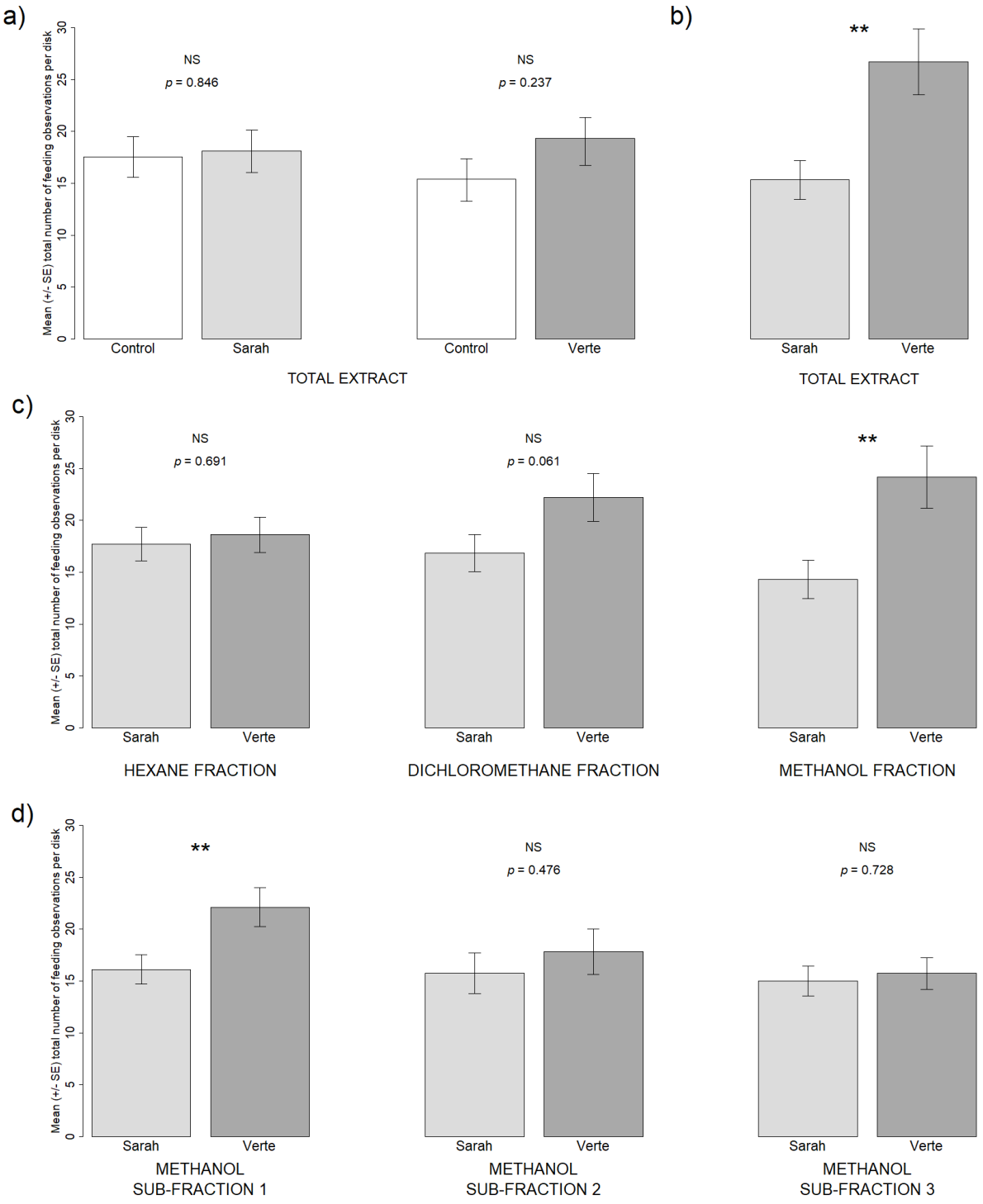

Fig. 5 Mean (± SE) total number of feeding observations per agar disk either supplemented with solvents only (Control) or (a) the total root extract of *S. alba* (cv. Sarah and Verte), (b) the total root extract of each cultivar, (c) one fraction of the total extract of the two cultivars, (d) one sub-fraction of the methanol fraction of the two cultivars. NS: $p > 0.05$, **: $p < 0.01$. N = 30 per experiment except for (a, left and middle) where N = 20. See behavioral kinetics in Fig. S5. Note that the agar disks are supplemented with sugar in this experiment.

DISCUSSION

Mechanisms underpinning host-plant selection by belowground insects is a key issue that remains understudied in chemical ecology. Studies on the effect of internal and root surface metabolites are scarce and rarely conducted among several species and several populations, which would however allow distinguishing species-specific from common cues shared by the whole host range. This study shows that the process of host acceptance in belowground larvae of a specialist species can be mediated by different cues at the intra- and interspecific scales.

Larval root consumption experiments on whole plants suggested that differences in development success may find some origin in the acceptance or rejection of the plants by larvae. Indeed, roots of all cultivars identified by Menacer *et al.* (2021) as favorable for larval development were unambiguously consumed. On the contrary, no significant difference in root length could be observed between infested and uninfested plants of the resistant cultivar of *S. alba* (*i.e.* Sarah). Although a rapid root regrowth after consumption, as observed in other plant species (Steinger and Müller-Schärer, 1992; Robert *et al.*, 2015, Hervé and Erb, 2019), might also explain this result, it still suggests that the poor development success could be due to the rejection of the plant soon after larvae started feeding. These results open the question of the cues that trigger acceptance and rejection of the different plants tested.

Feeding tests on agar disks revealed that total root extracts of *B. oleracea* and *B. rapa* stimulate larval feeding. No differences in feeding stimulation were observed between the two *B. rapa* cultivars, suggesting that the contrasts in larval performance observed in Menacer *et al.* (2021) are likely due to differences in root quality (*e.g.* nutrient content). Results obtained on the two plants species show that feeding stimulation is not due to a single compound, but rather to a synergistic action of several compounds. Indeed, a combination of fractions or subfractions is necessary to reproduce the stimulant effect of the total root extracts. In addition, polar compounds appear to be crucial in this synergy as the methanolic fraction is mandatory for behavioral effects to be induced. Polar compounds may include, for example, sugars or short-chained fatty acids, which are mostly known to have phagostimulant effects on root-feeding insects (Crombie and Darrah, 1947; Bernklau and Bjostad, 2005; Johnson and Nielsen, 2012). Some studies on belowground insects have demonstrated the synergistic effect of sugars with other less polar molecules in stimulating feeding. For example, Bernklau and Bjostad (2008) showed that in the western corn rootworm, feeding stimulation is initiated by the combined presence of sugars and lipids, each of which not being active alone. Furthermore, and although these studies are still rare, the current literature suggests that feeding stimulation by specialized metabolites occurs in specialist root herbivores, as in specialist aboveground ones (Bjostad and Hibbard, 1992; Robert *et al.*, 2012; Johnson, 2013). Since *D. radicum* larvae are specialist of brassicaceous plants, it is likely that certain specialized compounds such as glucosinolates, which are polar and mediate host recognition in females (Finch and Skinner, 1982; Tuttle *et al.*, 1988), could also stimulate larval feeding.

Feeding tests confronting total root extracts of *S. alba* to control disks did not show significant differences in larval feeding stimulation, although those confronting the two cultivars of this species did. These ambiguous results may suggest that the resistant cultivar does not contain deterrent compounds. However, it cannot be ruled out that a deterrent effect might be masked by the presence of sucrose in the agar disks, which is known to be highly stimulant for insect feeding (Chapman, 2003). Such masking effects have been demonstrated, for example, in the western corn rootworm, where the addition of a mixture of phagostimulant compounds overcame the normally deterrent effect of the insecticide thiamethoxam (Bernklau and Bjostad, 2008; Bernklau *et al.*, 2011). Moreover, the ability of sugars to mask the effect of deterrent compounds has also been demonstrated for aboveground herbivores (Shields and Mitchell, 1995; Cocco and Glendinning, 2012). Another hypothesis that could explain the feeding contrast observed between the two cultivars is the absence of feeding stimulants in root extracts of the less-preferred, resistant cultivar. Indeed, the absence of such stimulant compounds could lead to the non-acceptance of the plant, which has already been shown in some aboveground species (*e.g.* Reifenrath *et al.*, 2005). Whatever the origin of the intraspecific contrast within *S. alba* (*i.e.* deterrence of absence of stimulation), the bioguided fractionation approach demonstrated that the compounds involved in this contrast are very polar, since the only subfraction reproducing the original contrast is the most polar, water subfraction. An ambiguous effect was also observed with the semi-polar dichloromethane fraction, which might be due to a non-exclusive fractionation process. Water-soluble organic compounds are known to be crucial for host acceptance in various insect species (Johnson, 2013). Such compounds include many primary metabolites, *ca.* 80 % of those tested having phagostimulant effects (Erb *et al.*, 2013). Key stimulant ones might be less concentrated in the resistant *S. alba* cultivar. Water-soluble compounds may also include specialized metabolites, many of which having phagodeterrent effects (Brown and Gange, 1990; Johnson and Gregory, 2006; Movva and Pathipati, 2017). Some of these might be more concentrated in the resistant cultivar. Metabolomic analyses are now needed to identify candidate compounds which biological effect on larvae could be tested in feeding tests on agar disks.

Although Menacer *et al.* (2021) demonstrated a positive correlation between female oviposition preferences and offspring performance in *B. rapa*, a negative correlation was shown in *S. alba*. Thus, females lay more eggs on cultivars that are the most unfavorable for larval development in the latter species. The present study adds to these results by showing that host-plant recognition processes in larvae are involved, at least partly, in the development contrasts observed within *S. alba*. It further raises questions about the opposite effect of plant cues used for the recognition of this species by females and larvae. In aboveground insect species, females as well as larvae use both volatile (Bruce *et al.*, 2005; Liu *et al.*, 2016; Piesik *et al.*, 2009, 2013, 2018) and contact chemical compounds (Cervantes *et al.*, 2002; Fernández *et al.*, 2019; Riederer and Müller, 2006) as cues to select their hosts. Since belowground larvae must rely on root exudates and contact compounds to identify their hosts (Johnson, 2013), they

may use different cues compared to aerial females. A mismatch between cues used by females and larvae could therefore be at the origin of the discrepancy between female preference and larval performance, especially if the production of these cues relies on independent metabolic pathways.

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Supplementary informations

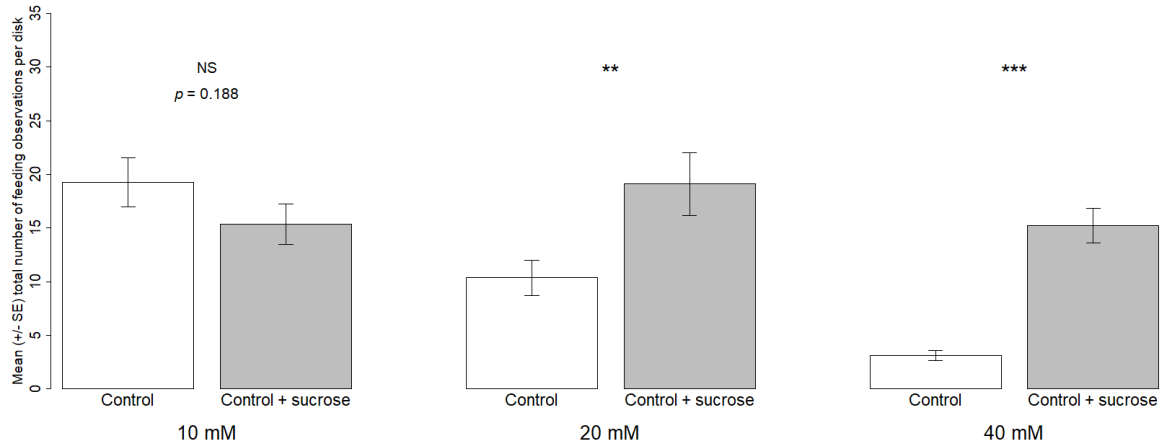


Fig. S1 Mean (± SE) total number of feeding observations per agar disk, either pure or supplemented with sucrose (grey) or not (white) at different concentrations. NS: $p > 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

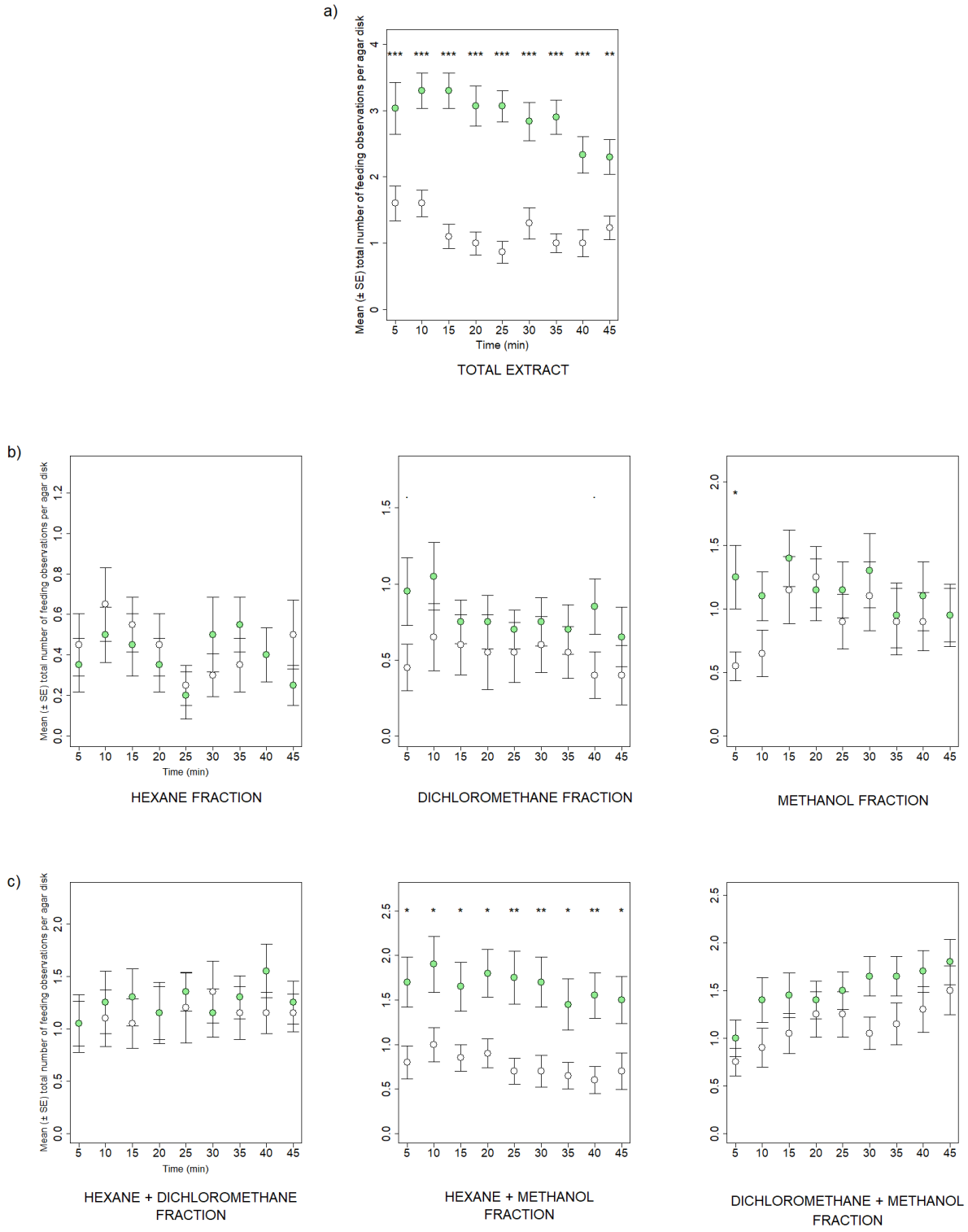


Fig. S2 Mean (± SE) total number of feeding observations per agar disk according to time either supplemented with (a) total root extract of *B. oleracea* or not, (b) one fraction of the total extract or not, (c) two fractions of the total extract or not. NS: $p > 0.1$, . : $p < 0.1$,*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Light green dots: cv. Parthenon, white dots: control

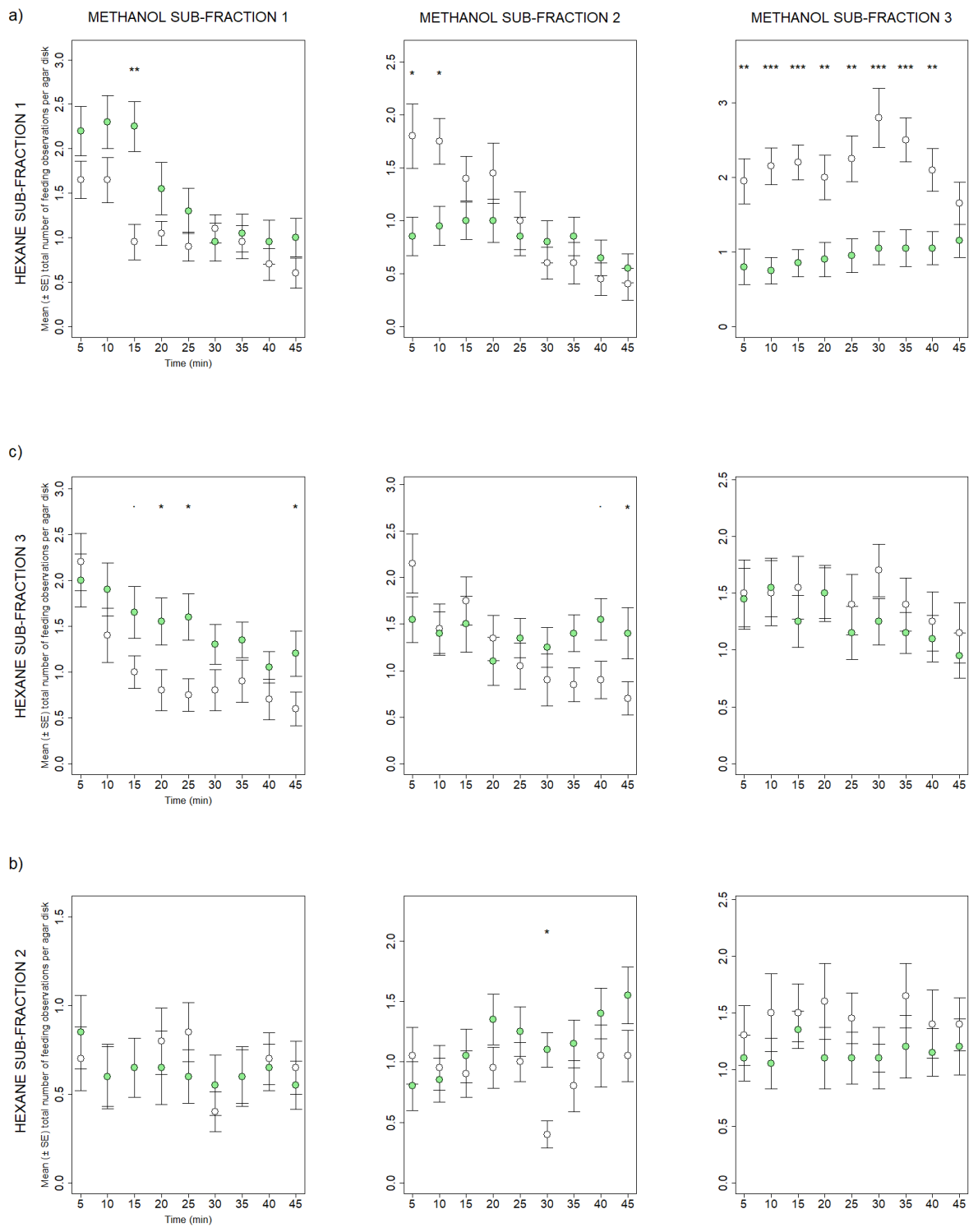


Fig. S3 Mean (± SE) total number of feeding observations per agar disk according to time either supplemented with (a) hexane sub-fraction 1 + one of each methanol sub-fraction, (b) hexane sub-fraction 2 + one of each methanol sub-fraction, (c) hexane sub-fraction 3 + one of each methanol sub-fraction of the root extract of *B. oleracea*. NS: $p > 0.1$, . : $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Light green dots: cv. Richi, white dots: control

Table S1 Statistics associated with the mean (± SE) total number of feeding observations per agar disk either supplemented with solvents only (Control) or combination of each hexane sub-fraction with each methanol sub-fraction of the root extract of *B. oleracea*. Significant *P*-values are indicated in bold.

		methanol								
		sub-fraction 1			sub-fraction 2			sub-fraction 3		
		χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
hexane	sub-fraction 1	4.7	1	**0.03**	1.27	1	0.259	17.43	1	**< 0.001**
	sub-fraction 2	0.04	1	0.849	2.04	1	0.153	1.04	1	0.308
	sub-fraction 3	5.04	1	**0.025**	0.44	1	0.509	0.38	1	0.54

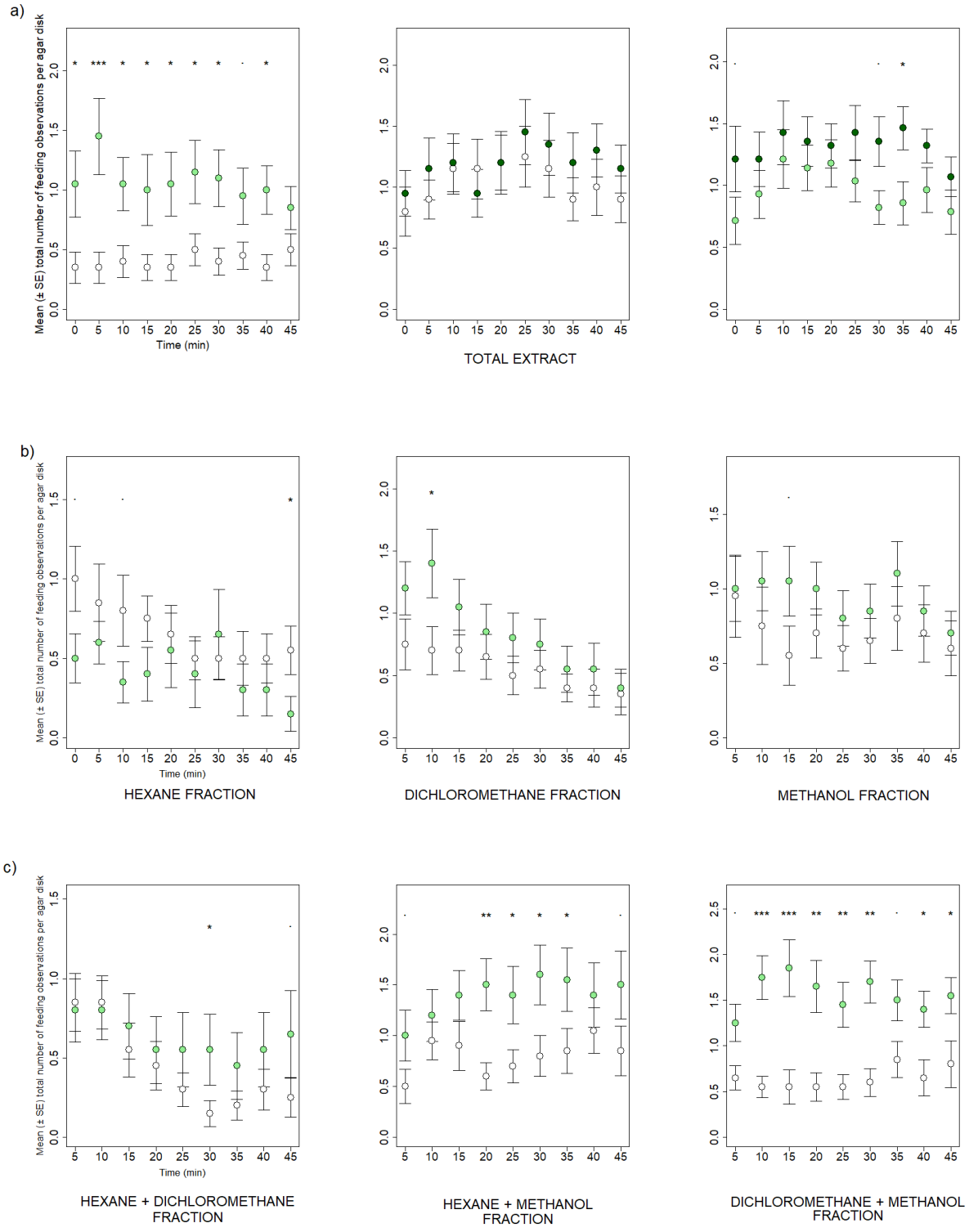


Fig. S4 Mean (± SE) total number of feeding observations per agar disk according to time either supplemented with (a) total root extract of *B. rapa* or not (left and middle) or total root extract of the two cultivars (right), (b) one fraction of the total extract or not, (c) two fractions of the total extract or not. NS: $p > 0.1$, . : $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Light green dots: cv. Richi, dark green dots: cv. Tabaluga, white dots: control

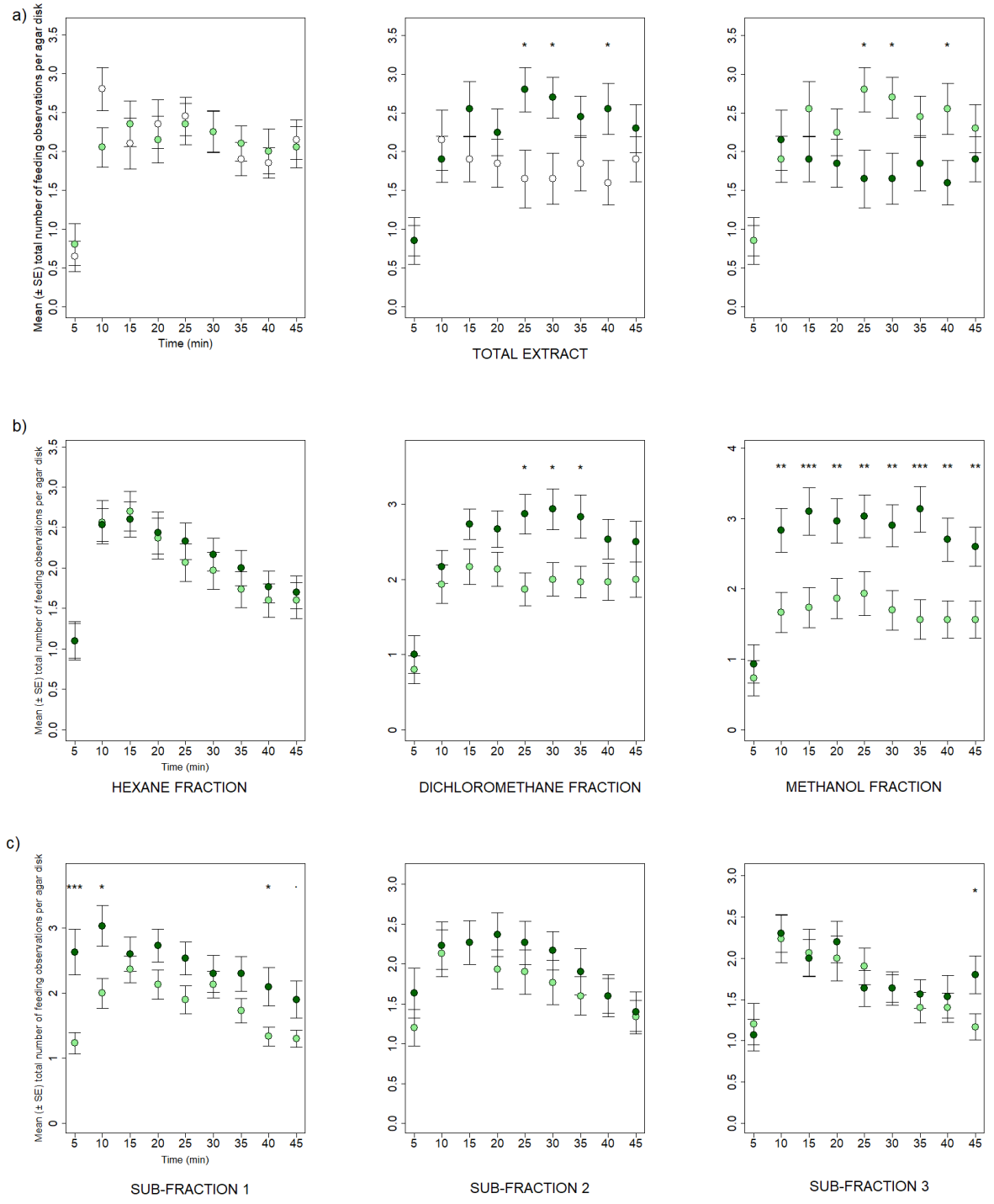
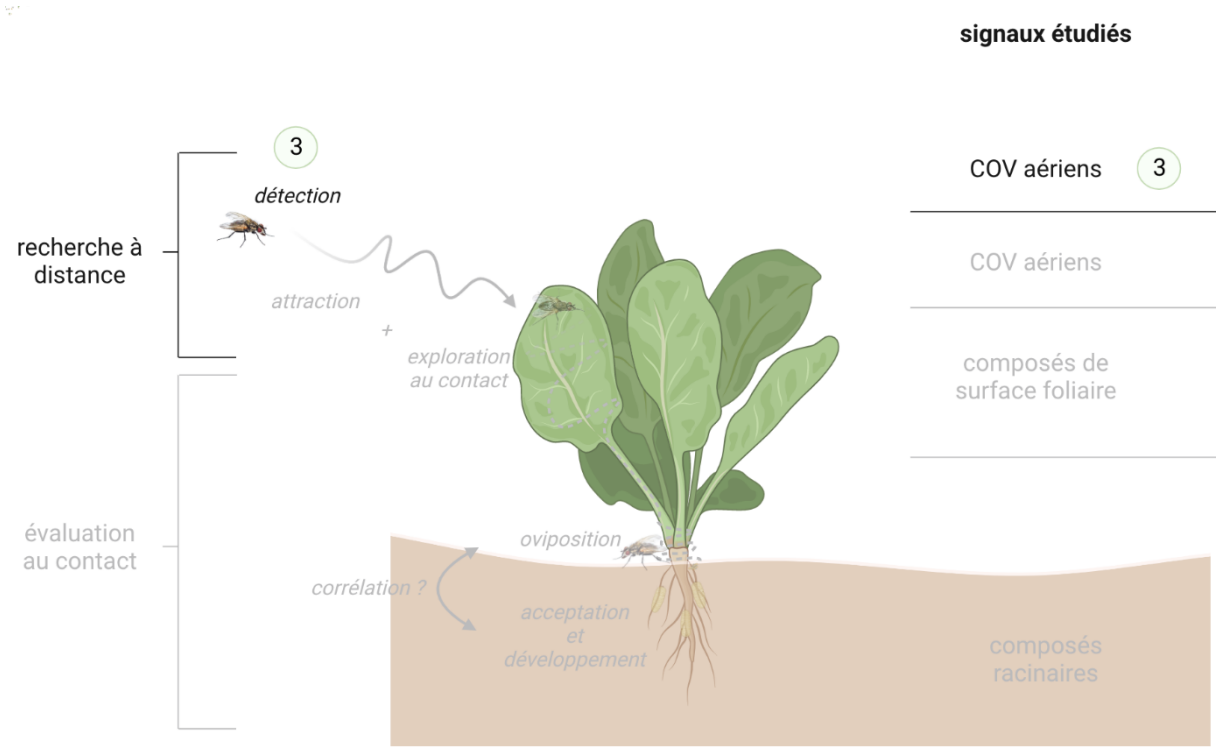


Fig. S5 Mean (± SE) total number of feeding observations per agar disk according to time either supplemented with (a) total root extract of *S. alba* or not (left and middle) or total root extract of the two cultivars (right), (b) one fraction of the total extract of the two cultivars, (c) one sub-fraction of the methanol fraction of the two cultivars. NS: $p > 0.1$, . : $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Light green dots: cv. Sarah, dark green dots: cv. Verte, white dots: control

Article 3

Sex- and maturity-dependent antennal detection of host plant volatiles in the cabbage root fly, *Delia radicum*



Sex and maturity-dependent antennal detection of host plant volatiles in the cabbage root fly, *Delia radicum*

Manuscript ready for submission in Journal of Insect Physiology

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Highlights

- Response thresholds and dose-response curves vary between sexes and maturity status
- Mature insect antennae respond more to several host-derived compounds
- Immature insect antennae respond more to a behaviourally attractive flower volatile
- Male and female antennae respond with different sensitivity to certain compounds

Abstract

Adult insect behaviour in response to plant-emitted volatile compounds varies between the sexes and as a function of maturity. These differences in behavioural responses can be due to modulation in the peripheral or central nervous system. In the cabbage root fly, *Delia radicum*, behavioural effects of certain host plant volatiles on female behaviour have been evaluated, and a large number of compounds emitted by brassicaceous host plants have been identified. We investigated here if the antennal detection of individual volatile compounds emitted by intact and damaged host plants differs between male and female, as well as immature and mature flies, using electroantennogram recordings. Our results showed dose-dependent responses to all tested compounds for mature and immature males and females. Mean response amplitudes varied significantly between sexes for three compounds, and between maturity

states for six compounds. For some additional compounds significant differences occurred only for high stimulus doses (interaction between dose and sex and/or dose and maturity status). Multivariate analysis revealed a significant global effect of maturity on electroantennogram response amplitudes and for one experimental session also a significant global effect of the sex. Interestingly, allyl isothiocyanate, a compound stimulating oviposition behaviour, elicited stronger responses in mature than in immature flies, whereas ethylacetophenone, an attractive flower volatile, elicited stronger responses in immature than in mature flies, which correlates with the behavioural role of these compounds. Several host-derived compounds elicited stronger responses in females than in males and, at least at high doses, stronger responses in mature than in immature flies, indicating differential antennal sensitivity to behaviourally active compounds. Six compounds did not cause any significant differences in responses between the different groups of flies. Our results thus confirm peripheral plasticity in plant volatile detection in the cabbage root fly and provide a basis for future behavioural investigations on the function of individual plant compounds.

Keywords: cabbage root fly (*Delia radicum*), olfactory plasticity, host plant volatiles, antennal sensitivity, electroantennogram

Introduction

Insect olfactory-guided behaviour is not only known to differ between males and females, but also to show strong plasticity as a function of adult maturity (age), and mating state (Gadenne et al., 2016). This plasticity helps insects to take optimal decisions for when responding to sex pheromones, host-, food-, or oviposition site odours. Freshly mated male moths do for example not respond to female-emitted sex pheromones, because they would not be able to mate successfully (Gadenne et al., 2001). Blood-feeding insects such as mosquitoes and hematophagous bugs also adapt their responses to host odours as a function of age and mating state, thus avoiding taking unnecessary risks during the approach and attack of a host animal ((Gadenne et al., 2016) and references therein).

More recently, differences in behavioural responses to plant volatiles as a function of sex, maturity and mating state have been investigated in various phytophagous insects. In the fruit fly *Bactrocera tyroni*, mated females were more attracted to their host fruit volatiles than virgin females (Devescovi et al., 2021). In the tomato fruit fly, *Neoceratitis cyanescens*, both mated and unmated females responded to ripe tomato fruits, whereas males were rather attracted by odours from unripe fruits (Brevault and Quilici, 2010). Females moth change their behavioural preferences from flower odours to odours emitted by plants used for oviposition after mating (Saveer et al., 2012). As a last example, male moths are only rarely attracted by host plant volatiles, but host plant volatiles can synergize responses to the female-

emitted sex pheromone (Masante-Roca et al., 2007; Ochieng et al., 2002; Reddy and Guerrero, 2004; Varela et al., 2011; Yang et al., 2004).

The physiological mechanisms underlying behavioural plasticity have been investigated in detail specifically in male moths responding to sex pheromones. Here, depending on the species, either changes in the peripheral or central olfactory system have been described ((Gadenne et al., 2016) and references therein). In the noctuid moth *Agrotis ipsilon*, male responses to the sex pheromone increase with maturity, but not responses to plant volatiles (Greiner, 2002). However central, but not peripheral neuron responses to a flower volatile increased after mating in *A. ipsilon*, possibly facilitating a switch from searching for a mate to feeding behaviour (Barrozo et al., 2011). In some moth species, on the contrary, both peripheral and central nervous responses to host plant volatiles have been found to vary between sexes and as a function of maturity and mating state, in line with behavioural changes. For example in the grapevine moth *Lobesia botrana*, antennal lobe neurons in unmated males and in mated females responded more often to selected plant compounds than in mated males and unmated females (Masante-Roca et al., 2007). Antennal responses of *Spodoptera littoralis* females to plant odours, on the other hand, were generally stronger in virgin females as compared to mated females (Martel et al., 2009). Interestingly, glomerular responses to a flower odour measured by calcium imaging activity representing essentially receptor neuron responses in the same species decreased after mating, whereas glomerular responses to host plant volatiles (cotton) increased after mating (Saveer et al., 2012). Finally, differential expression of antennal olfactory genes has been shown in different insects, such as *Drosophila suzukii*, and *Bactrocera dorsalis* after mating (Crava et al., 2019; Jin et al., 2017).

Adults of the cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae), feed from the nectar of a large diversity of plant species, whereas females are specialists of Brassicaceae plants for oviposition (Finch, 1989; Rännbäck, 2008). In addition to visual cues, females have been shown to use certain host plant-emitted odours, such as allyl isothiocyanate (AITC), to localize oviposition sites in the field (Tuttle et al., 1988). Also *cis*-3-hexenyl acetate, a volatile emitted by damaged host plants has been shown to be attractive for females (Kergunteuil et al., 2012). Other compounds such as dimethyl disulfide (DMDS) strongly decrease oviposition (Ferry et al., 2009; Kergunteuil et al., 2012; Lamy et al., 2017). Upon emergence and for the first few days of their life, unmated females and males search first for food sources and seem not to respond to host plant odours (Hawkes, 1975; Nottingham, 1988a). Females usually start oviposition after a maturation phase that lasts about five days after emergence (Hawkes, 1975; Nottingham, 1988b), but it is unknown whether this maturation, in addition to mating, contributes to changes in plant odour responses. Much less is known about how *D. radicum* males find their mating partners and if they use odour cues for orientation (Finch and Skinner, 1982). In particular, no sex pheromone has been identified so far. Males show some behavioural responses to AITC, but lower than females. This response could potentially help them to find a mating partner as this compound is only emitted by brassicaceous plants (Finch and Skinner, 1982). The main olfactory organ of *D. radicum*, the

funiculus of the antenna, carries abundant olfactory sensilla (Ross and Anderson, 1991, 1987). Interestingly, female antennae carry about 40 % more sensilla than male antennae and lower sensillum numbers in males were observed for all types, which correlates with behavioural differences between sexes (Ross and Anderson, 1987).

In this study, our aim was to investigate whether the antennal detection of key volatiles identified from intact and damaged host plants varies as a function of sex and adult maturity in *D. radicum*, and could thus explain differences in odour-guided behaviours. We investigated peripheral responses of male and female individuals to potentially behaviourally-relevant plant volatiles, using electroantennographic recordings. Additionally to comparing dose-response curves and mean response amplitudes between sexes, we also compared between freshly hatched immature and mature, mated insects.

Materials and Methods

Experimental insects

Delia radicum L. (Diptera: Anthomyiidae) originated from a laboratory rearing initially constituted from a field population collected in the field in 2019 (Pleumeur-Gautier, Brittany, France). Flies were reared on rutabagas as described in Neveu Bernard-Griffiths (1998) and fed with a milk powder: yeast: sugar (1:1:1) mixture. For EAG experiments, pupae were transferred to small cages, which were changed every two days to a new cage, controlling thus for age of emerged insects. Males and females were kept together to obtain mated mature males and females, which were fed *ad libitum* as under rearing conditions. Mature, mated insects were used at five to seven days of age. Immature, virgin insects were obtained by transferring individual pupae into Eppendorf cups with a small amount of food and a moist piece of cotton. Emerging insects were checked every day and insects were used within 24 h after emergence.

EAG recording

Whole insects were used for EAG recordings, with flies slipped into a plastic disposable pipette tip with the head and antennae protruding, secured with dental wax. Glass pipettes filled with Beadle Ephrussi Ringer were used as electrodes. The reference electrode was inserted into a small opening in the cuticle of the eye and the recording electrode was placed tightly in the center of the undamaged funiculus similarly to EAG recordings in other flies (e.g. Hieu et al., 2014). The electrodes were connected to an amplifier (axoclamp 2B, Molecular Devices, San Jose, CA, USA), and an IDAC-4 device to digitize signals (Syntech, Kirchzarten, Germany). EAG Pro software (Syntech, Kirchzarten, Germany) was used to record the antennal responses.

Stimulation procedure

The antennae were superfused by a constant air-stream (0.3 $m.s^{-1}$) through a glass tube. Stimuli were applied onto a piece of filter paper inserted into a Pasteur Pipette and the pipette was introduced into the glass tube through a hole in the sidewall. A stimulus controller (CS 55, Syntech, Kirchzarten, Germany) was used to apply a 500 ms air pulse through the stimulation pipette. Inter-stimulus intervals lasted at least 1 s. For each stimulus, increasing doses in decadic steps from 0.01 to 1000 µg were applied to obtain dose-response curves and the solvent, mineral oil, was applied before and after each stimulus series. The mean response to mineral oil before and after a stimulus dose series was subtracted from each amplitude value (= net amplitude) to compensate for amplitude differences between insects and over time. Recordings from fly antennae were, however, extremely stable over several hours, so that we did not need to further normalize data as a function of time. For each stimulus, sex, and maturity state 10 individuals were tested per session (see below).

Volatile organic compounds used for antennal stimulation

All compounds tested were previously identified in odour blends emitted by host plants of *D. radicum*. Fifteen compounds were retained, which were taken as representatives for a variety of chemical classes having demonstrated effects on phytophagous insects in general and specialists of Brassicaceae in particular: sulfur compounds (AITC, DMDS), green leaf volatiles (*cis*-3-hexenyl acetate), defense hormones (methyl salicylate), aromatic compounds (ethylacetophenone), sesquiterpenes (α-farnesene and β-caryophyllene) and monoterpenes (α-pinene, α-thujene, eucalyptol, limonene, linalool, myrcene, ocimene and sabinene). Some of these compounds have been identified as having effects on mature *D. radicum* females. Indeed, AITC and cis-3-hexenyl acetate have been shown to attract mature flies toward oviposition sites (Finch and Skinner, 1982; Kergunteuil et al., 2012; Nottingham, 1988b; Tuttle et al., 1988) and to stimulate oviposition in field (Kergunteuil et al., 2012; Lamy et al., 2018) and laboratory conditions (Traynier, 1967, 1965). DMDS (Ferry et al., 2009; Kergunteuil et al., 2012; Lamy et al., 2018, 2017), eucalyptol (Lamy et al., 2017) and limonene (Kergunteuil, 2013) were all found to reduce oviposition. Moreover, ethylacetophenone is emitted by flowers of many species including *Lobularia maritima*, a brassicaceous species whose flowers are particularly attractive to *D. radicum* females (Kergunteuil et al., 2012; Rännbäck, 2008), and methyl salicylate is a herbivore-induced plant volatile emitted by damaged brassicaceous plants in particular (Pierre et al., 2011). All other tested compounds are found in the VOC profiles of brassicaceous plants, but have not been tested for their effect on behavioural responses of *D. radicum* adults yet (Kergunteuil et al., 2015; Pierre et al., 2011; Tollsten and Bergstrom, 1988). Compounds were tested in two experimental sessions separated by a few weeks, with different individuals used in each session. Nine compounds were tested in the first session only, three compounds were tested in the second session only, and three compounds were tested in both

sessions to ensure consistency in the interpretation of the results of the two sessions (Supplementary Table 1).

Data processing and statistical analyses

All analyses were performed using the R software version 4.1.3 (R Core Team 2022). EAG amplitude responses were analyzed both univariately and multivariately. First, for each compound, the mean amplitude response was analyzed using Wald tests applied to Linear Mixed Models in which the sex, maturity status and dose (log-transformed), as well as all interactions between these variables, were considered as independent variables and the individual was considered as a random factor (R packages 'car' (Fox and Weisberg, 2019), 'lme4' (Bates et al., 2015), 'RVAideMemoire' (Hervé, 2022)). Responses were log-transformed to ensure model fit and, since some negative responses were obtained, normalized by computing $x + |\min(x)| + 0.01$ to avoid zeroes that would cause infinite logs. Pairwise comparisons of Estimated Marginal Means (EMMeans) were computed with p-values adjusted using the False Discovery Rate correction (Benjamini & Hochberg 1995) (R package 'emmeans' (Lenth et al., 2022)). The multivariate approach consisted in two Redundancy Analyses (RDAs, R package 'vegan' (Oksanen *et al.*, 2022)), i.e. one per session. Responses were the overall mean EAG amplitude per individual and per compound (i.e. all doses confounded), which were normalized as above for consistency with the univariate approach and autoscaled. Independent variables were the sex, the maturity status and the interaction between these two factors. The effect of these factors was tested using a permutation F test with 9 999 permutations (Legendre and Legendre 2012). Pairwise comparisons were performed also using permutation F-tests and p-values corrected with the False Discovery Rate method.

Results

EAG response characteristics and dose-response curves vary between tested compounds

Antennae of *D. radicum* responded in a dose-dependent manner to all tested compounds (Table 1, Supplementary Figure 1). The EAG amplitudes of responses varied between individual insects and reached maximum net amplitudes of up to 10 mV, for the highest tested dose for certain compounds in some insects. The time course of responses differed between different compounds independently of the sex and maturity (Figure 1a). AITC for example provoked large and narrow EAG signals, whereas ethylacetophenone elicited smaller maximum EAG amplitudes, but much longer-lasting responses (Figure 1a). Similarly, dose-response curves with different thresholds and slopes were found for different compounds (Table 1). Generally, antennae responded already at the lowest tested dose to ocimene, β-caryophyllene and DMDS, but responses increased only slightly when increasing the tested

doses (Fig. 1b). For other compounds such as eucalyptol and α-pinene, responses only started at a dose of 100 µg and increased at a dose of 1 000 µg (Fig. 1b). For still other compounds such as linalool and AITC, responses started at 1 or 10 µg and increased steeply at high doses (Fig. 1b).

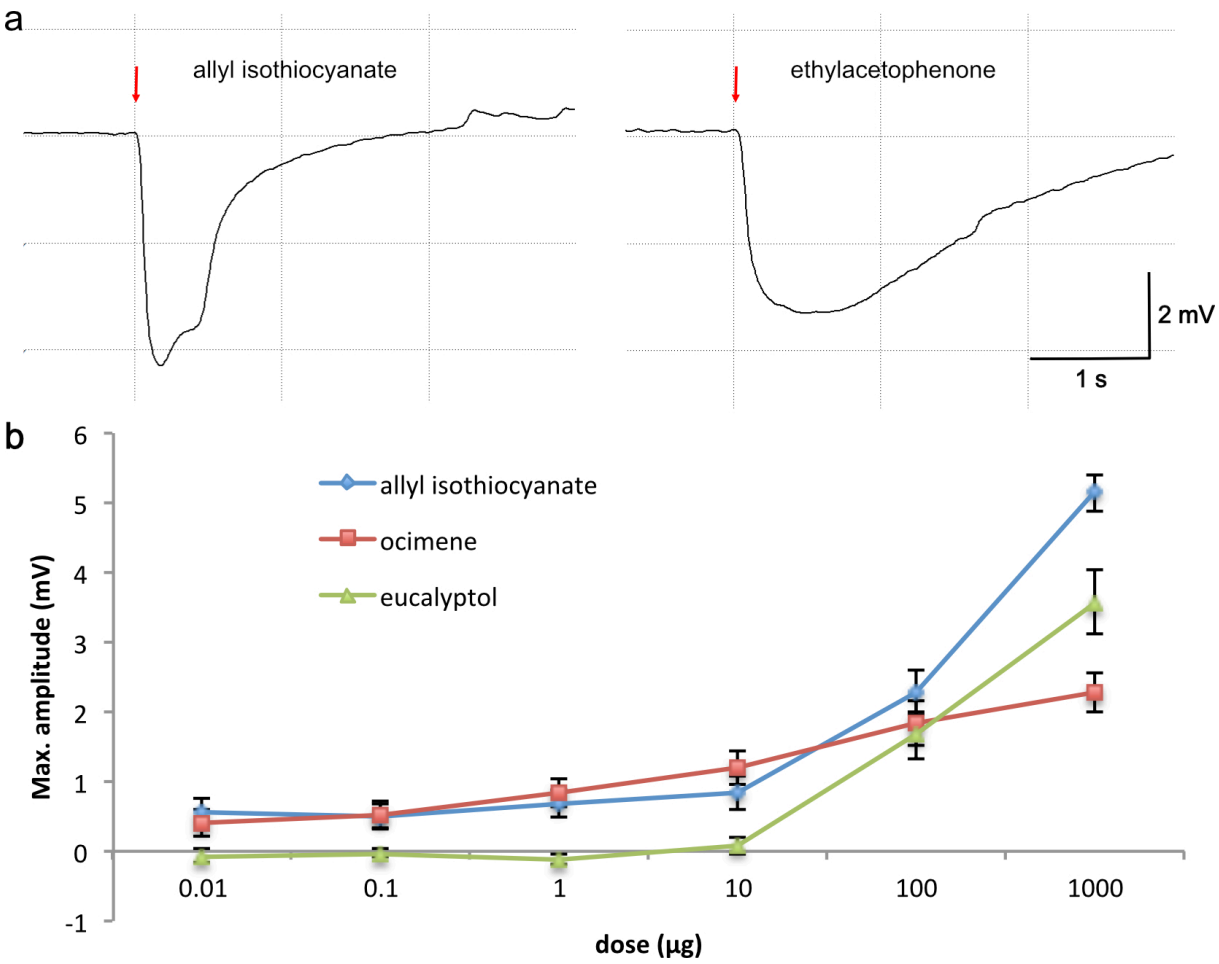


Fig. 1 Examples for characteristic EAG responses to different plant compounds in mature *Delia radicum* females. **a)** Time course of EAG responses to allyl isothiocyanate and ethylacetophenone at the 1000 µg dose. Note that amplitudes over time and duration of the EAG responses differ strongly between the two stimuli. **b)** Examples for dose-response curves (mean amplitude, ± SE, n=10) for stimulation with allyl isothiocyanate, ocimene and eucalyptol. Note the differences in thresholds and slopes as a function of the applied stimuli.

EAG responses to a majority of compounds are sex- and maturity-dependent

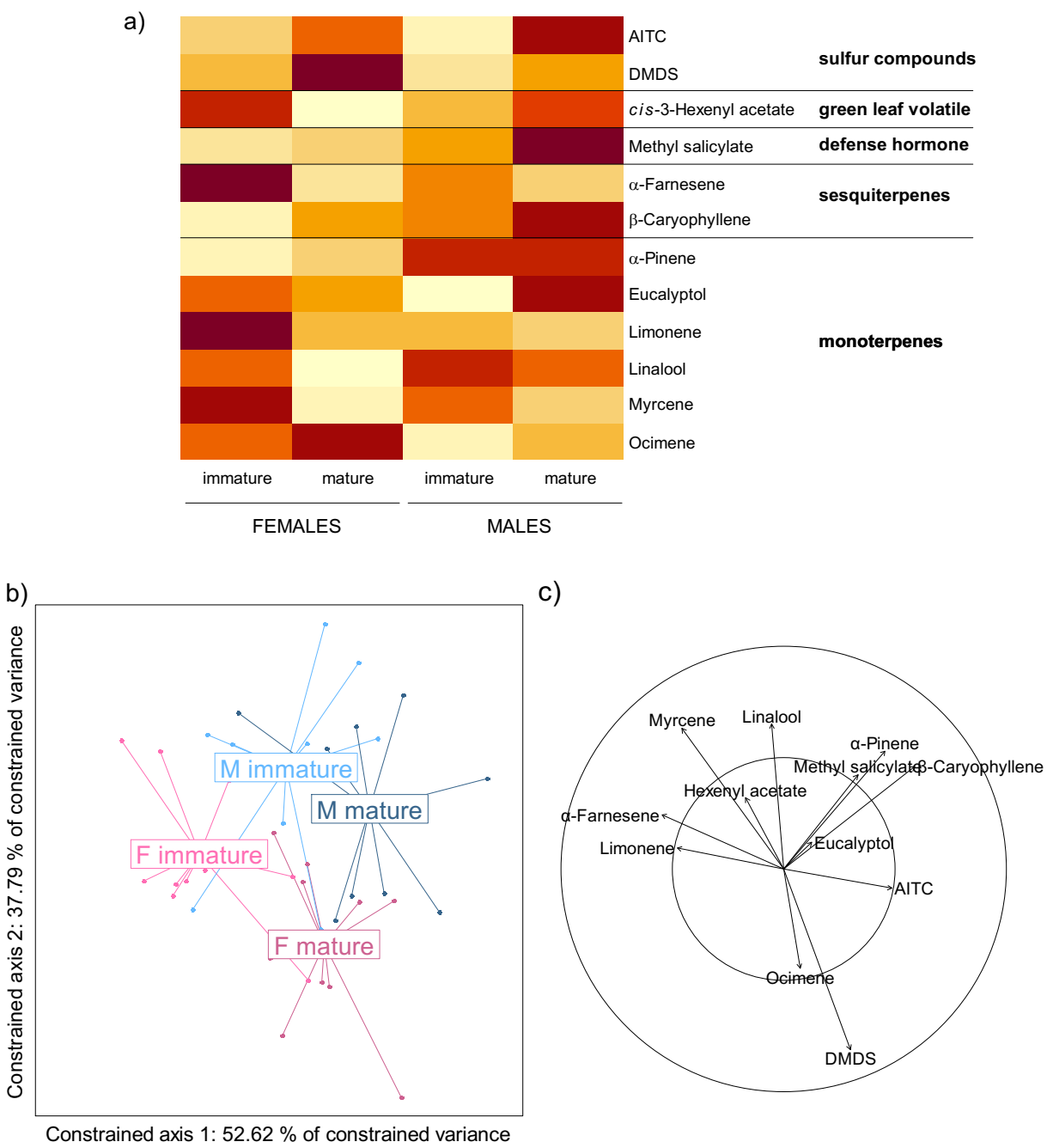
Mean amplitudes of responses, as well as slopes of the dose-response curves, varied significantly between sexes, maturity status or both for 10 of the 15 compounds tested (Table 1). These effects were very similar between the two experimental sessions and are therefore described together.

An interaction of dose and sex was only significant for two of the 15 compounds tested (Table 1). In males larger responses were found at high stimulus doses for eucalyptol than in females. In females, on the other hand, larger responses were found at high stimulus doses for myrcene than in males. An interaction of dose and maturity was significant for five compounds (Table 1). Responses at high doses of AITC, *cis*-3-hexenyl acetate, limonene, myrcene and ocimene exhibited larger amplitudes in mature compared to immature flies.

When focusing on the mean amplitude of response per compound, both the univariate and multivariate approaches gave a consistent picture (Table 1, Figure 2). In the first test session, a significant global effect of the sex was observed ($F = 2.57$, $p = 0.019$, Figure 3b). Male antennae respond more intensely to methyl salicylate, β-caryophyllene and α-pinene (Figures 3a,c). An independent, non-interacting ($F = 0.72$, $p = 0.703$), effect of the maturity status was observed ($F = 2.86$, $p = 0.008$, Figure 3b). Antennae of immature individuals responded more strongly to α-farnesene, limonene, and myrcene, in contrast to mature individuals who responded more strongly to AITC and DMDS (Figures 3a,c). In the second session, only a significant effect of the maturity status was observed ($F = 4.48$, $p = 0.004$, Figure 3e), with a stronger antennal response of immature individuals to ethylacetophenone and α-thujene, while mature individuals responded more strongly to AITC (Fig 3d,f). No significant effect of the sex or the interaction between the sex and the maturity status was observed in the second session (respectively $F = 0.22$, $p = 0.165$; $F = 1.01$, $p = 0.395$) (Figure 3d,f).

Table 1: Wald tests on the effect of sex, maturity status, dose as well as all interactions between these variables on the mean amplitude antennal response of Delia radicum for the compounds tested according to sessions.

	Compound	Dose			Sex			Maturity			Dose:Sex			Dose:Maturity			Sex:Maturity			Dose:Sex:Maturity		
		χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P
Session 1	AITC	128,53	1	< **0,001**	0,42	1	0,519	0,05	1	0,824	0,05	1	0,815	8,22	1	**0,004**	2,16	1	0,142	1,85	1	0,174
	DMDS	14,76	1	< **0,001**	1,68	1	0,195	7,78	1	**0,005**	0,07	1	0,797	1,74	1	0,187	0,94	1	0,333	0,31	1	0,579
	cis-3-hexenyl acetate	254,43	1	< **0,001**	0,01	1	0,918	2,21	1	0,137	0,23	1	0,630	8,63	1	**0,003**	0,86	1	0,354	0,11	1	0,743
	Methyl salicylate	162,23	1	< **0,001**	1,08	1	0,298	3,39	1	0,065	0,94	1	0,331	0,06	1	0,805	0,74	1	0,391	2,33	1	0,127
	α-Farnesene	77,08	1	< **0,001**	0,08	1	0,775	6,19	1	**0,013**	1,53	1	0,216	1,85	1	0,174	2,23	1	0,135	0,51	1	0,477
	β-Caryophyllene	48,25	1	< **0,001**	9,98	1	**0,002**	6,92	1	**0,009**	0,46	1	0,498	2,25	1	0,133	0,02	1	0,879	0,18	1	0,674
	α-Pinene	99,68	1	< **0,001**	3,64	1	0,056	0,09	1	0,767	0,74	1	0,389	2,74	1	0,098	0,03	1	0,870	1,08	1	0,299
	Eucayptol	159,40	1	< **0,001**	0,92	1	0,336	0,08	1	0,777	4,34	1	**0,037**	1,65	1	0,198	1,09	1	0,296	1,52	1	0,217
	Limonene	140,33	1	< **0,001**	1,96	1	0,162	3,48	1	0,062	0,12	1	0,726	5,84	1	**0,016**	0,37	1	0,541	0,02	1	0,882
	Linalool	379,59	1	< **0,001**	0,69	1	0,406	0,28	1	0,598	0,95	1	0,329	0,28	1	0,597	0,83	1	0,364	1,19	1	0,276
	Myrcene	144,22	1	< **0,001**	0,06	1	0,799	5,42	1	**0,020**	10,31	1	**0,001**	6,15	1	**0,013**	0,74	1	0,389	1,63	1	0,202
	Ocimene	124,60	1	< **0,001**	4,69	1	**0,030**	2,05	1	0,152	0,10	1	0,752	4,35	1	**0,037**	0,01	1	0,928	0,64	1	0,423
Session 2	AITC	214,44	1	< **0,001**	0,38	1	0,537	7,46	1	**0,006**	0,04	1	0,848	1,10	1	0,294	2,22	1	0,136	2,16	1	0,142
	Ethylacetophenone	386,01	1	< **0,001**	0,13	1	0,714	6,44	1	**0,011**	2,31	1	0,128	0,21	1	0,647	0,08	1	0,782	0,01	1	0,934
	α -Thujene	47,81	1	< **0,001**	0,07	1	0,789	2,16	1	0,141	0,01	1	0,939	0,06	1	0,804	0,10	1	0,758	0,20	1	0,651
	Myrcene	158,87	1	< **0,001**	14,05	1	< **0,001**	6,11	1	**0,013**	6,09	1	**0,014**	1,82	1	0,177	0,02	1	0,883	0,85	1	0,357
	Ocimene	215,26	1	< **0,001**	3,44	1	0,064	0,80	1	0,372	3,58	1	0,059	0,28	1	0,597	0,05	1	0,828	0,85	1	0,356
	Sabinene	216,50	1	< **0,001**	2,59	1	0,107	0,07	1	0,790	0,03	1	0,855	0,10	1	0,746	2,11	1	0,146	1,08	1	0,300


a)
AITC
DMDS
cis-3-Hexenyl acetate
Methyl salicylate
α-Farnesene
β-Caryophyllene
α-Pinene
Eucalyptol
Limonene
Linalool
Myrcene
Ocimene
sulfur compounds
green leaf volatile
defense hormone
sesquiterpenes
monoterpenes
immature
mature
immature
mature
FEMALES
MALES
b)
M immature
M mature
F immature
F mature
Constrained axis 2: 37.79 % of constrained variance
Constrained axis 1: 52.62 % of constrained variance
c)
Myrcene
Linalool
α-Pinene
Methyl salicylate
β-Caryophyllene
Hexenyl acetate
α-Farnesene
Limonene
Eucalyptol
AITC
Ocimene
DMDS

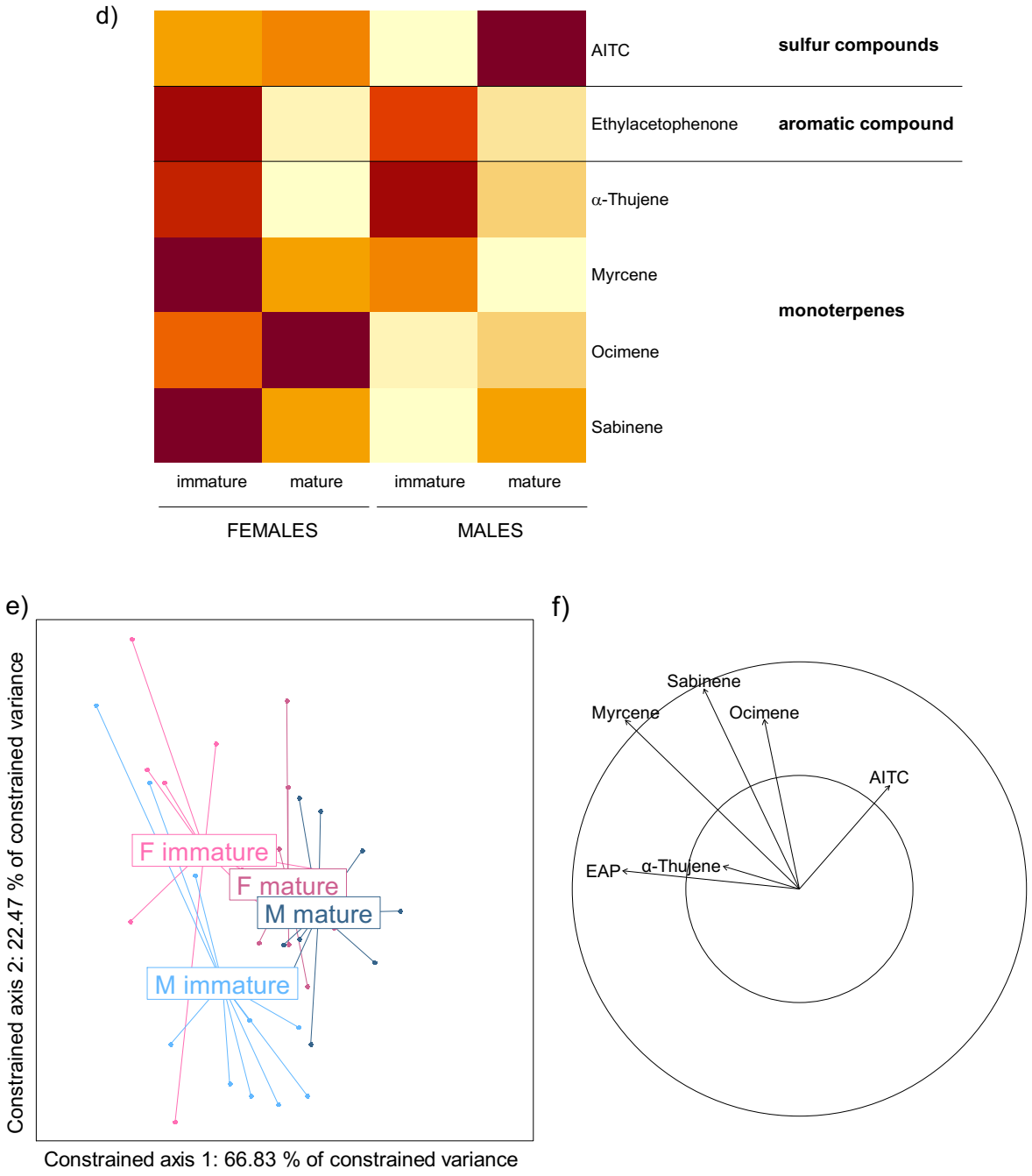


Fig. 3 Sex and maturity-dependent EAG responses to plant volatile compounds. Heatmaps of mean EAG amplitudes in response to the tested compounds according to sex and maturity of *Delia radicum* individuals, for session 1 (a) and session 2 (d). Mean amplitudes are pictured independently for each compound and represented by a color gradient from light yellow (lowest level) to dark red (highest level). Note that results are not comparable between lines, due to compound-specific statistical standardisation of mean response amplitudes. Score plots and associated correlation circles of the redundancy analyses performed on mean EAG response amplitudes for each individual, for session 1 (b)(c) and session 2 (e)(f). EAP: ethylacetophenone.

Discussion

Our results show highly variable responses to plant-derived compounds, as a function of sex and maturity. Interestingly, the differences in antennal responses between mature and immature flies, as well as between males and females, depend strongly on the tested compound. Behavioural significance of the tested compounds is known only for a few of them and almost exclusively in mature females so far.

Two of the three compounds, known to reduce oviposition in *D. radicum* females, DMDS and limonene, elicited stronger responses in mature than in immature flies, at least at high doses. The observed reduced oviposition rate of flies exposed to these compounds (Lamy et al., 2017; Liu et al., 2018) might thus be due to a high sensitivity to these compounds, mediating a potential repulsive effect. However, behavioural assays with immature insects need to confirm whether the sensitivity difference is indeed responsible for such an effect only in mature insects. Allyl isothiocyanate and *cis*-3-hexenyl acetate, compounds known to attract flies towards oviposition sites (Traynier 1965, 1967, Städler, 1978), elicited increased EAG responses, at least at high doses, in mature compared to immature adults. Whereas increased sensitivity in mature females correlates with the stimulation of oviposition, it would now be interesting to evaluate behavioural effects of these compounds on mature males. A compound emitted by flowers used for nectar feeding in *D. radicum* (Rännbäck, 2008), ethylacetophenone, elicited much stronger EAG responses in immature insects than in mature insects. This is correlated with the importance to find food sources in immature flies. Interestingly many of the compounds shown to be emitted in various host plants of *D. radicum* elicited stronger responses in mature than in immature insects (at least at higher doses) and some compounds elicited stronger responses in females than in males at higher doses. This increased sensitivity could indicate that the concerned compounds might play a role in host finding behaviour. However, the occasionally observed higher sensitivity in males needs to be further investigated.

Our results on the plasticity of antennal responses to host plant odours in this species provide a complex dataset, allowing to choose compounds of interest for future behavioural experiments as a function of sex and maturity of this insect. However, even though the compounds not eliciting any differences in antennal responses between sexes and maturity might be potentially of less importance for adult flies, one should not exclude these compounds from further investigations, because modulation of plant odour sensitivity might well occur at the central nervous level, as previously observed in different moth species (Barrozo et al., 2011; Masante-Roca et al., 2007).

Differences in antennal olfactory sensitivity to plant-derived volatiles between mature and immature insects and between the sexes have rarely been investigated systematically. In other studies, single doses of different components have been used as stimuli (Martel et al., 2009), but this limits the conclusions that can be drawn. Indeed, as shown by our results, dose-response curves with different thresholds and different slopes can occur and thus differences can be hidden when testing only a single dose.

Nevertheless significant differences in responses to a panel of plant-derived compounds have been found in females of the noctuid moth *Spodoptera littoralis* as a function of mating and adult age (Martel et al., 2009). Also in the Mediterranean fruit fly, *Ceratitis capitata*, EAG responses to plant headspace of different host plants varied between the sexes, but also in both males and females as a function of mating state and the origin of the flies (laboratory-reared or wildtype) (Sollai et al., 2020). EAG responses of female blowflies to attractive and repellent compounds similarly depended on the maturity: response amplitudes increased with age (Crnjar et al., 1990). Our results on the cabbage root fly confirm that indeed differences in plant volatile responses between the sexes and maturity of insects exist already at the detection level.

Whereas modulatory mechanisms of central olfactory sensitivity have been described in different insects, among others as a function of maturity (Gadenne et al., 2016; Ignell et al., 2001; Jarriault et al., 2009), the mechanisms underlying peripheral modulation of olfactory sensitivity are not well understood. Up- and down- regulation of the expression of olfaction-related antennal genes, such as olfactory receptors and odorant binding proteins, for example, seem to correlate with antennal sensitivity. In the oriental fruit fly *Bactrocera dorsalis*, mating leads to changes in the expression of various olfactory receptor genes, including ORs, GRs and IRs, but the corresponding ligands are not known (Jin et al., 2017). In the same species, changes of EAG sensitivity to a compound present in host plants as a function of starvation depended on the presence of short neuropeptide F and the expression of its receptor (Jiang et al., 2017). With the recently sequenced genome of *D. radicum* available (Sontowski et al., 2022), it will be possible in the future to identify olfactory genes involved in the differences in antennal sensitivity between the sexes and as a function of maturity. Another potential mechanism is modulation of peripheral olfactory sensitivity by modulatory feedback neurons from the brain and reaching the antennae, as it has been shown for serotoninergic neurons in mosquitoes (Siju et al., 2008). Immunostaining of *D. radicum* antennae would be necessary to potentially reveal such a mechanism.

Credit author contribution statement

Kathleen Menacer: Conceptualization, Formal analysis, Writing**. Maxime R. Hervé:** Conceptualization, Formal analysis, Writing, supervision, Project administration, Funding acquisition. **Anne-Marie Cortesero:** Conceptualization, Writing, supervision, Funding acquisition. **Tom Aujames:** Investigation**. Sylvia Anton:** Conceptualization, Methodology, Investigation, Formal analysis, Writing-original draft, supervision.

Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that would have appeared to influence the work reported in this paper.

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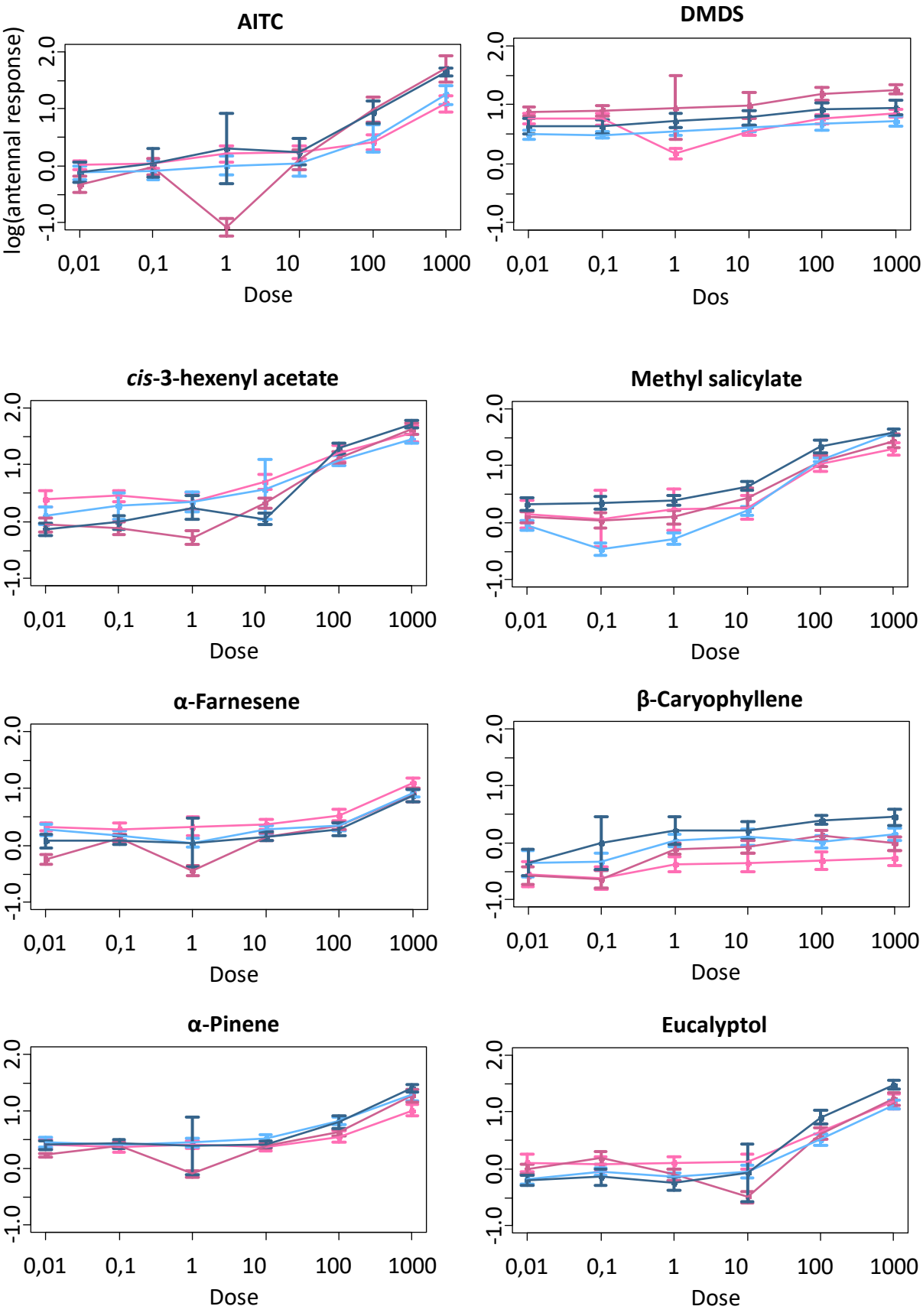
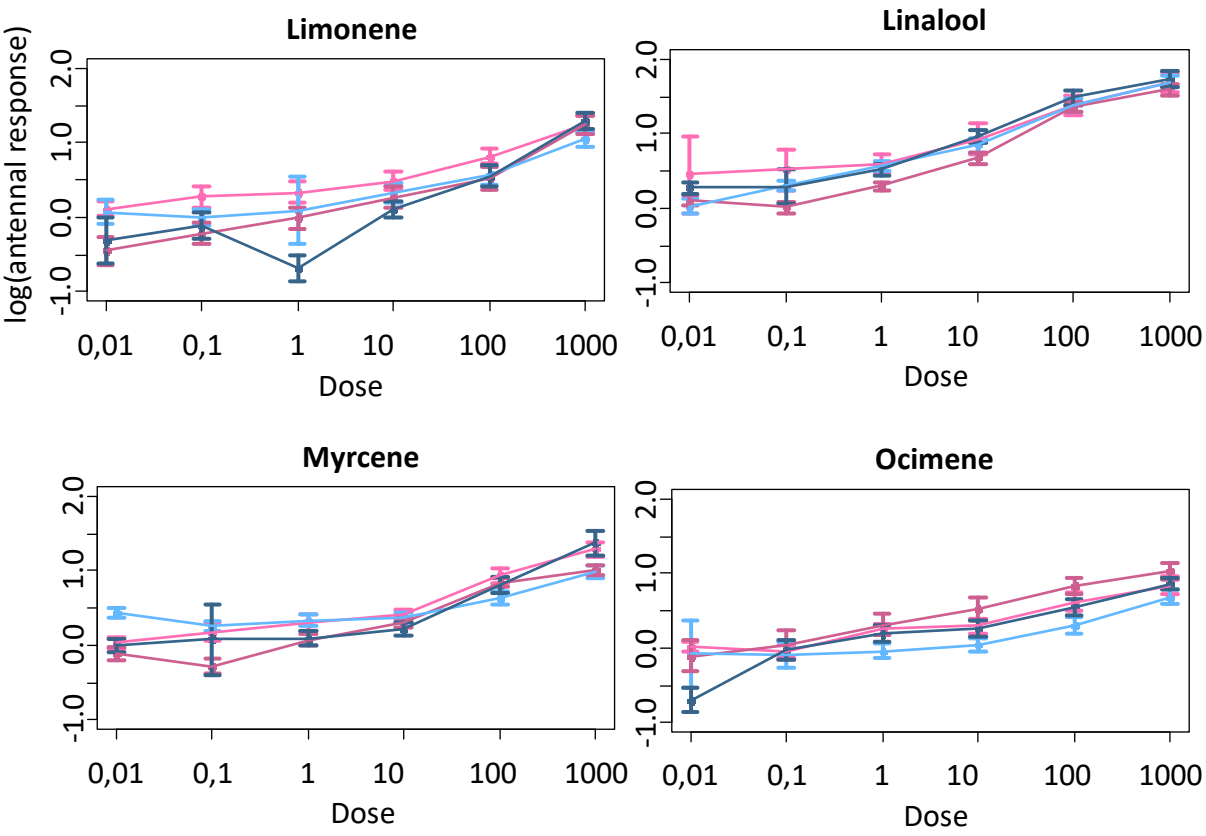
Table 1 Compounds used for stimulation in EAG experiments.

Compound	Chemical class	purity	Purchased from*	Ecological relevance	References
Allyl isothiocyanate	sulfur compound	≥ 95%	Aldrich	Attractive to gravid females found in some crucifer COV profiles stimulates oviposition	(Wallbank & Wheatley, 1976; Finch, 1978; Finch & Skinner, 1982; Nottingham, 1988; Tuttle et al., 1988; Liu et al., 2018)
DMDS	sulfur compound	≥ 99%	Aldrich	Reduces oviposition found in some Brassica COV profiles	(Wallbank & Wheatley, 1976; Pierre et al., 2011; Kergunteuil et al., 2012; Lamy et al., 2017; Liu et al., 2018)
cis-3-Hexenyl acetate	green leaf volatile	≥ 98%	Sigma-Aldrich	Main compound of attractive Chinese cabbage and found in other crucifers	(Wallbank & Wheatley, 1976; Tollsten & Bergstrom, 1988; Pierre et al., 2011; Kergunteuil et al., 2012; Liu et al., 2018)
Methyl salicylate	defense hormone	≥ 99%	Sigma-Aldrich	HIPV and attracting insects in the field in different crops, including Brassica	(Orre et al., 2010; Simpson et al., 2011; Pierre et al., 2011; Kergunteuil et al., 2012)
Ethylacetophenone	aromatic compound	97%	Sigma Aldrich	Flower-derived compound, attractive as food signal	(Rännbäck, 2008)
α-Farnesene	sesquiterpene	≥ 90%	Bedoukian Bio	Found in the volatile profiles of different *D. radicum* Brassica host species and predominant in the VOC profile of broccoli	(Tollsten & Bergstrom, 1988; Pierre et al., 2011; Kergunteuil et al., 2015)
β-Caryophyllene	sesquiterpene	≥ 98.5%	Sigma	Found in the volatile profiles of different *D. radicum* Brassica hosts species and predominant in the VOC profile of oil seed rape	(Tollsten & Bergstrom, 1988; Pierre et al., 2011; Kergunteuil et al., 2015)
α-Pinene	monoterpene	98%	Aldrich	Found in the VOC profiles of different *D. radicum* Brassica hosts species	(Tollsten & Bergstrom, 1988; Pierre et al., 2011; Kergunteuil et al., 2015)
α-Thujene	monoterpene	95 %	Toronto Research Chemicals	Found in the VOC profiles of different *D. radicum* Brassica hosts species	(Kergunteuil et al., 2015)
Eucalyptol	monoterpene	99%	Aldrich	Reduces oviposition found in some crucifer VOC profiles	(Lamy et al., 2017; Liu et al., 2018)
Limonene	monoterpene	97%	Sigma Aldrich	Found in volatile profiles of different *D. radicum* Brassica hosts species, reduces plant colonization	(Pierre et al., 2011; Kergunteuil, 2013; Kergunteuil et al., 2015; Liu et al., 2018)
Linalool	monoterpene	97%	Aldrich	Predominant in the VOC profile of Chinese cabbage	(Kergunteuil et al., 2015)
Myrcene	monoterpene	Technical grade	Aldrich	Found in the volatile profiles of different *D. radicum* Brassica host species	(Tollsten & Bergstrom, 1988; Kergunteuil et al., 2015)
Ocimene (mixture of isomers)	monoterpene	≥ 90%	Sigma-Aldrich	Found in the volatile profiles of different *D. radicum* Brassica hosts species	(Tollsten & Bergstrom, 1988)
Sabinene	monoterpene	98%	abcr chemicals	Found in the volatile profiles of different *D. radicum* Brassica hosts species	(Tollsten & Bergstrom, 1988)

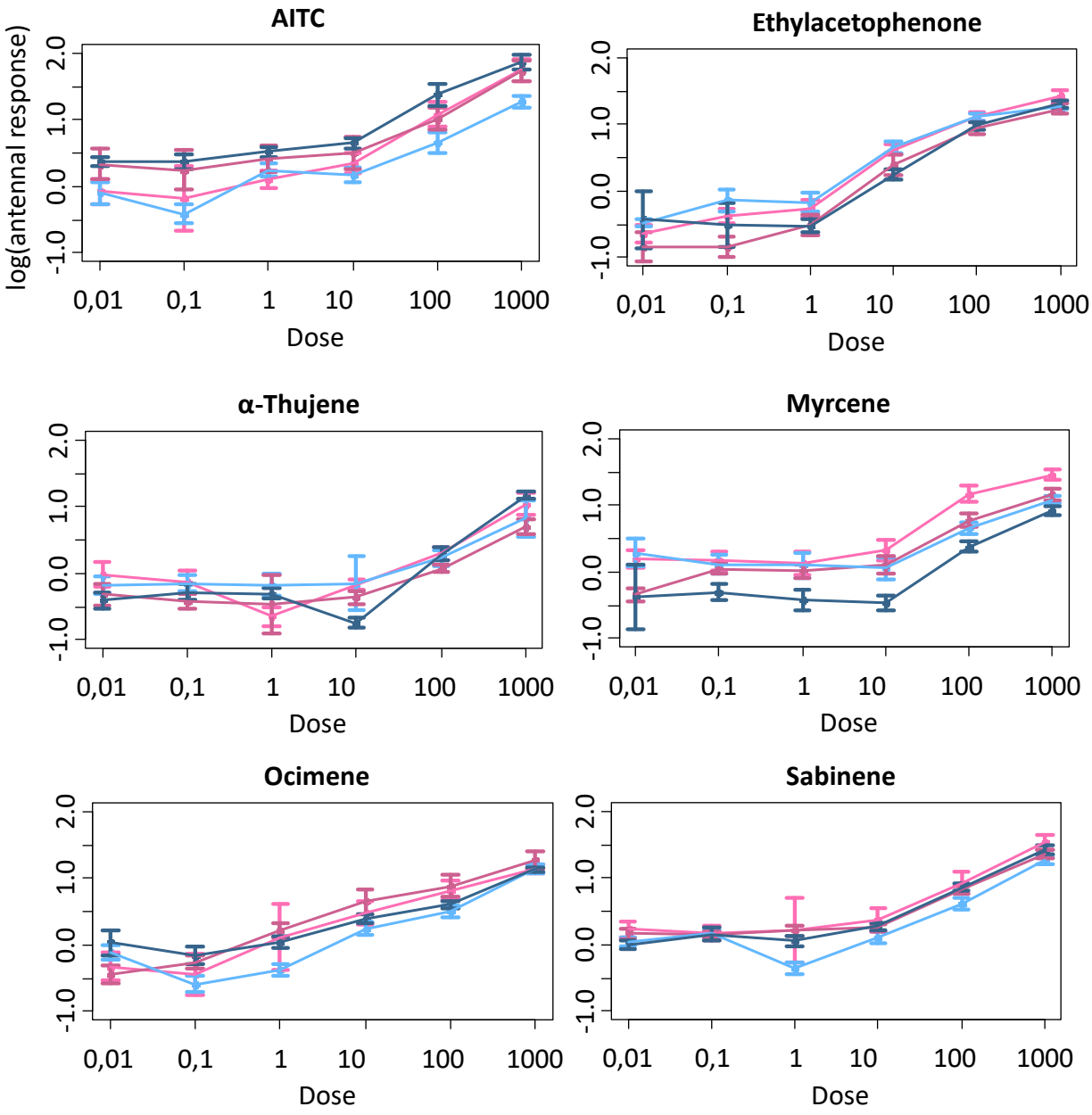
* suppliers: Bedoukian, Danbury, CT, United States; Sigma-Aldrich, Saint Quentin Fallavier, France; Toronto Research Chemicals, Toronto, Canada; abcr chemicals, Karlsruhe, Germany

Supplementary Figure 1 Dose-response curves of antennal detection by *D. radicum* individuals of the different VOCs tested in session 1. Dark blue = mature males, light blue = immature males, dark pink = mature females, light pink = immature females. AITC: allyl isothiocyanate; DMDS: dimethyl disulfide



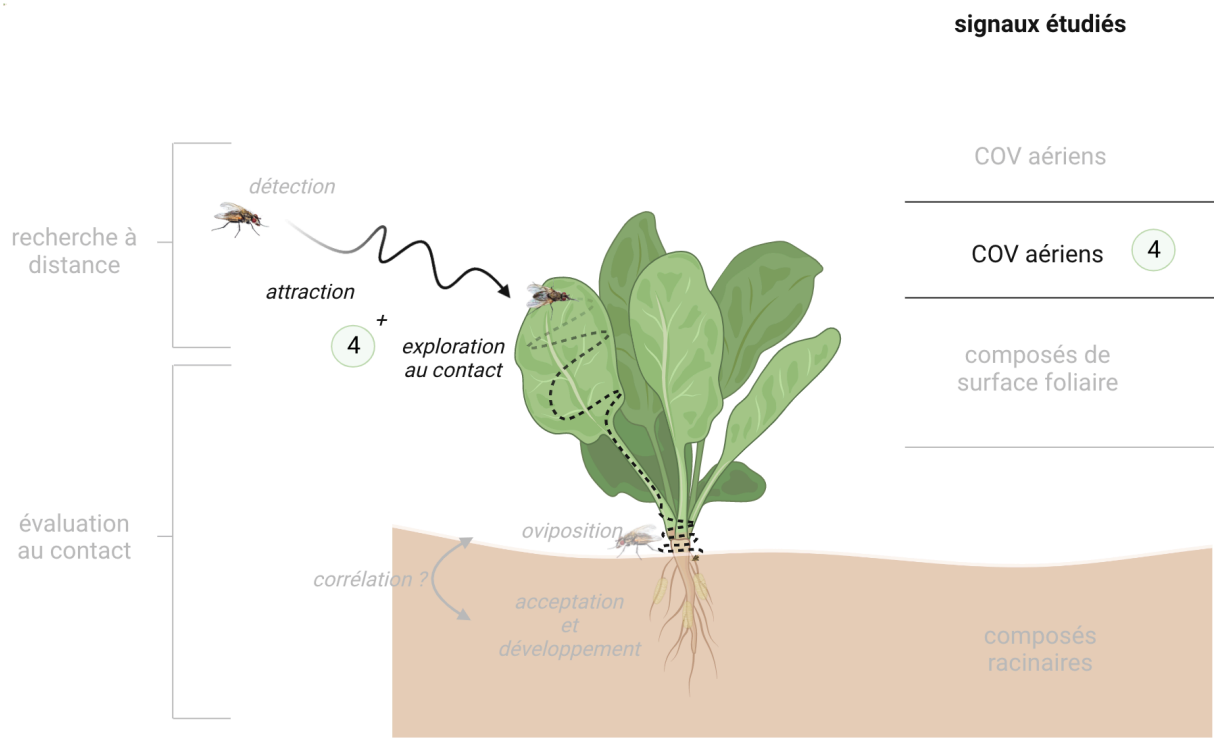

Limonene
Linalool
Myrcene
Ocimene
log(antennal response)
-1.0
0.0
1.0
2.0
0,01
0,1
1
10
100
1000
Dose

Supplementary Figure 2 Dose-response curves of antennal detection of *D. radicum* individuals to each volatile organic compound tested in the second experimental session (mean SE values). Dark blue = mature males, light blue = immature males, dark pink = mature females, light pink = immature females. AITC: allyl isothiocyanate



Article 4

Host plant selection in phytophagous insects: volatiles can act independently at distance and at contact



Host plant selection in phytophagous insects: volatiles can act independently at distance and at contact

Manuscript ready for submission in Oecologia

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Anne Marie Cortesero and Maxime Hervé equal contribution last author

ABSTRACT

Although volatile organic compounds (VOCs) are known to play a key role in distance host location in phytophagous insects, they can also influence insects' behavior once at contact. However, studies on this second aspect are still very limited. Furthermore, in such studies distance effects are rarely distinguished from true contact effects. In the cabbage root fly, *Delia radicum*, VOCs are known to mediate both female attraction and oviposition. Previous studies have shown a contrast in attractiveness and oviposition between three host species, namely *Brassica oleracea*, *B. rapa* and *Sinapis alba*. In this study, we aimed to determine VOCs that mediate these effects, and to distinguish whether their effect applies at distance or at contact. To do this, headspace collections of VOCs emitted by the three plant species, as well as two populations of *B. rapa* and *S. alba* contrasted for oviposition, were performed and analyzed in combination with electroantennodetection, to identify compounds detected by females' antennae. The behavioral effect of the electrophysiologically-active compounds was assessed using an artificial leaf device with synthetic VOCs. Our results show that compounds detected by females are ubiquitous VOCs which combinations are species specific, and that although one compound has behavioral effects alone, most effects are triggered by mixtures of several VOCs. Furthermore, we show that all behaviorally-active VOCs or mixtures have effects either at distance or at contact, not both. This pleads for a more systematic distinction of effects that apply at distance or at contact, whatever these are all mediated by VOCs.

Keywords: volatile organic compounds; exploration behavior stimulation; distance effect; *Delia radicum*

INTRODUCTION

Plants emit complex mixtures of volatile compounds that may contain up to a hundred different molecules (Dudareva *et al.*, 2006). These volatile organic compounds (VOCs) strongly influence ecological interactions between plants and insects, as the latter rely highly on olfaction to grasp their external environment (Krieger and Breer, 1999, Carrasco *et al.*, 2015). Each plant species, and at a finer scale each population or even genotype, emits VOCs in particular amounts and ratios, such that not two ever exhibit strictly identical VOC blends (Rajabaskar *et al.*, 2013; Ahmed *et al.*, 2019). These differences are used by phytophagous insects to identify and select their host plants. From an evolutionary perspective, there is thus a strong selection pressure on insects to develop efficient ways to detect host-specific blends, on which the insect survival and reproduction depend (Bruce and Pickett, 2011). Although attraction can be induced by one or a few taxonomically characteristic compounds, such as observed with specific VOCs of Brassicaceae (Nottingham *et al.*, 1991; Blight *et al.*, 1995; Baoyu *et al.*, 2001), Asteraceae (Krasnoff and Dussourd 1989), Rosaceae (Knight and Light, 2001) or Alliaceae (Judd and Borden, 1989), insects of several orders have been shown to possess olfactory receptors able to detect ubiquitous VOCs such as fatty acid derivatives, phenylpropanoids and isoprenoids (Hansson *et al.*, 1999; Bruce *et al.*, 2005). Studies have shown that host recognition is mostly based on the detection of a host-specific combination of either specific or ubiquitous VOCs, rather than on the detection of a single host-specific compound. As an example, Webster *et al.* (2008a, 2008b) observed that a blend of 15 non-host specific VOCs emitted by *Vicia faba* elicited the same attraction for *Aphis fabae* as the emissions of an intact plant. It was further shown that the combination of these VOCs was necessary for attraction to be triggered, and that 10 of the compounds were even repulsive when tested alone (Webster *et al.*, 2010). Many examples in Diptera (Robacker *et al.*, 1992; Zhu *et al.*, 2003; Birkett *et al.*, 2004; Alagarmalai *et al.*, 2009), Lepidoptera (Honda *et al.*, 1998; Bruce and Cork, 2001; Natale *et al.*, 2003; Riffell *et al.*, 2009a, 2009b) also show that a combination of VOCs can be significantly more attractive than compounds taken individually.

Although VOCs are known to play a key role in distance host location (Bruce *et al.*, 2005), they can also act synergistically with contact compounds and influence the insect behavior once in contact with the plant (Gouinguené *et al.*, 2005; Braccini *et al.*, 2015). Furthermore, it has been shown in many species such as *Bombyx mori* (Damodaram *et al.*, 2014), *Drosophila melanogaster* (Dweck *et al.*, 2013), *Bactrocera dorsalis* (Kamala Jayanthi *et al.*, 2014a; Roh *et al.*, 2021) and *Anopheles arabiensis* and *A. coluzzii* (Asmare *et al.*, 2017), that certain VOCs alone or in mixtures can stimulate female oviposition on their own and thus be important in host selection at contact. As an example, Müller and Hilker (2001) tested the effect of VOCs on the behavior of the monophagous chrysomelid *Cassida stigmatica*, by comparing *Tanacetum vulgare* petioles wrapped with perforated filter paper tubes to dummy petioles. They observed that walking duration was longer on *T. vulgare* petioles than on dummy ones, showing that VOCs alone can influence host assessment and thus trigger contact exploration behaviors. However,

in most examples where the effect of VOCs on host selection is investigated, the distance effect (i.e. attraction of the insect to the oviposition site) and contact effect (i.e. stimulation of exploration or oviposition once in contact) are poorly distinguished, which does not allow to understand precise cues involved at each step of the host selection behavioral sequence.

In this study, we aimed to (i) identify VOCs involved in host recognition by females of the cabbage root fly *Delia radicum*, and (ii) determine their involvement in either female attraction at distance or stimulation of host pre-ovipositional exploration behavior. In this species, receptive females exhibit a stereotypical host exploration behavior before accepting a host plant as a suitable oviposition site (Zohren, 1968), which involves several types of cues, both physical and chemical. Physical cues such as color, shape or texture (Brown and Anderson 1996; Roessingh and Stadler, 1990; Tuttle *et al.*, 1988), as well as contact chemical cues (Städler and Schöni 1990; Baur *et al.*, 1996; Braven *et al.*, 1996; Chilcott 1997; de Jong *et al.*, 2000; Gouinguené and Städler, 2006) have been extensively studied. Other studies have shown the involvement of plant VOCs in mediating the female behavior (Finch, 1978; Finch and Skinner, 1982; Hawkes and Coaker, 1979). It has been shown, for example, that allylisothiocyanate is involved in long-distance attraction (Hawkes and Coaker 1979; Wallbank and Wheatley 1979) while other VOCs such as dimethyl disulfide (Lamy *et al.*, 2017) or *cis*-3 hexenyl acetate (Kergunteuil *et al.*, 2012; Lamy *et al.*, 2018) influence oviposition. Both VOCs and contact cues perceived simultaneously synergistically mediate host plant acceptance (de Jong and Städler,1999). In parallel, studies have shown contrasts in attractiveness between different host species (Kergunteuil *et al.*, 2015a, 2015b) and others have characterized VOCs released by these species (Wallbank and Wheatley, 1976; Tollsten and Bergström, 1988; Pierre *et al.*, 2011; Kergunteuil *et al.*, 2015a; Durenne *et al.*, 2018). However, to our knowledge, no studies have both characterized the compounds emitted by plants in *D. radicum*'s host range and identified those involved in either attracting females or stimulating their host exploration behavior prior to oviposition.

In the present study, experiments were conducted on three host species of *D. radicum*, namely *Brassica oleracea*, *B. rapa* and *Sinapis alba*, which are known to be contrasted both in terms of attractiveness (Kergunteuil *et al.*, 2015a, 2015b) and oviposition (Menacer *et al.*, 2021). At a finer scale, two populations (here, cultivars) of *B. rapa* and *S. alba*, also contrasted for oviposition (Menacer *et al.*, 2021), were included as well. These contrasts suggest fine-tuned detection and recognition abilities in *D. radicum* females, but the influence of volatiles on these discrimination abilities remains unknown. Headspace collection of VOCs emitted by the different host plants was performed and the compounds present in the blends were identified by GC-MS. In parallel, electroantennodetection was performed to identify, among all VOCs present in the blends, those that are detected by the females' antennae. Finally, the behavioral effect of the different compounds detected, both taken individually and in combination, was tested using an artificial leaf device with synthetic VOCs

MATERIALS AND METHODS

Plants

Experiments were carried out with five different cultivars, all of which being commercial: two cultivars of *Sinapis alba* ('Verte' and 'Sarah'), two cultivars of *Brassica rapa* ssp. *pekinensis* ('Richi' and 'Tabaluga') and one cultivar of *B. oleracea* ('Parthenon'). Single seeds were sown in individual 3.5 × 3.5 × 5 cm clumps filled with potting soil (Premier Tech Horticulture) and plants were grown in a climatic chamber at optimum conditions (20 ± 1 °C, 16L:8D and 70 ± 10 % RH) where they were watered twice a week with a nutritive solution (Hoagland and Arnon, 1950). One week before the experiments started, plants were repotted individually in 7 × 7 × 6.5 cm plastic pots containing the same substrate. All plants were at the 3-4 true leaves stage when used for experiments.

Insects

Females of *D. radicum* originated from a laboratory rearing initially constituted from a field population collected in the field in 2019 (Pleumeur-Gautier, Brittany, France). Flies were reared on rutabagas as described in Neveu Bernard-Griffiths (1998) and fed with a milk powder: yeast: sugar (1:1:1) mixture. One week after emergence, females were considered fertilized and used for experiments until they were 12 days old. Females were identified based on the sexual dimorphism in the size of the eye-spacing, which is much more pronounced in females than in males (Smith 1927).

Volatile collection

Volatiles of the five cultivars tested were collected using a dynamic headspace technique. The aboveground part of an individual plant was enclosed in a PET bag (35 x 43 cm, Alfapac), where an airflow was maintained thanks to two pumps (KNF, Neuberger) connected with PTFE tubing. The entrance and exit flow rates were regulated by flowmeters at 300 and 200 $ml.min^{-1}$, respectively, to create a positive pressure inside the bag and thereby prevent contaminations from the environment. An activated charcoal filter was placed between the upstream pump and the bag for the same reason. Volatiles were collected during 24 h on a cartridge filled with 30 mg of Porapak Q (80:100 mesh, Sigma-Aldrich) placed downstream from the sampling bag. After sampling, all cartridges were sealed with Parafilm®, wrapped into aluminum foil and stored at -20 °C until further use. Six to eight replicates were performed per cultivar.

Volatile identification and quantification

Samples were analysed at the "Platform for Chemical Analyses in Ecology" (PACE), with the support of the LabEx CeMEB (Centre Méditerranéen pour l'Environnement et la Biodiversité, Montpellier, France). Sampling cartridges were desorbed chemically using 200 µl of hexane (≥ 98.5 %; Carlo Erba reagents) and stored at -20 °C until analysis. Identification and semi-quantitative quantification of

volatiles was achieved using a Shimadzu QP2010Plus gas chromatograph-mass coupled to a quadrupole mass spectrometer (GC-MS, Shimadzu Scientific Instruments). The GC was equipped with an Optima 5-MS fused silica capillary column (length: 30 m; diameter: 0.25 mm; film thickness: 0.25 µm, Macherey-Nagel), with helium as carrier gas (1 $ml.min^{-1}$). The temperature ramp started at 40 °C hold for 1 min, then increased to 220 °C at 12 $°C.min^{-1}$, and finally hold for 2 min. One microliter of sample was injected into a splitless injector set at 200 °C. Mass spectra were recorded in electronic impact mode (EI) at 70 eV over a m/z mass ranging from 38 to 350. The temperature of the transfer line and the ion source were set at 250 °C. Data were processed according to Kidyoo *et al.* (2022) using the MZmine™ software version 2.53 (Pluskal *et al.*, 2010). Retention times of a series of *n*-alkanes (Alkanes standard solution, 04070, Sigma Aldrich®) were used to convert retention times into retention indices. Control samples collected the same week as the samples analyzed were used to subtract potential contaminant compounds from the samples. Compound identification was based on computer matching of mass spectra with a database (NIST 2005 MS library, Wiley 9th edition), on retention times and mass spectra of external synthetic standards, and on retention indices reported in the literature (Adams, 2007).

Electrophysiological recordings

Elutions from all samples of the same cultivar were pooled together and then concentrated under nitrogen flow for the recordings. Electroantennography assays were performed using *D. radicum* females only. Each female was immobilized with forceps, its head was cut and separated from the thorax, then put into an adapted glass capillary. It was obtained from glass capillary (length: 76 mm, diameter: 1.12 mm; World Precision Instrument) pulled and cut using a vertical micropipette-puller (P-30 model, World Precision Instruments). The capillary was previously filled with an electrolytic solution of Ringer (6.0 $g.l^{-1}$ NaCl, 0.4 $g.l^{-1}$ KCl, 0.3 $g.l^{-1}$ $CaCl_2$, 3 $g.l^{-1}$ $NaHCO_3$) and connected to a reference electrode. The tip of the antenna was in contact with the recording electrode, also filled with the electrolytic solution, at the opposite side of the reference electrode. A stimulus controller (CS-55 Syntech Ockenfels) was set to provide a continuous flow of purified and humidified air, passing through a glass tube to send the VOCs one by one to the antenna at a flow rate of 245 $ml.min^{-1}$. Electroantennography assays were performed using the coupled gas chromatography-electroantennographic detection method (GC-EAD). A volume of 2 µl of the solution was injected in a 8890 GC system gas chromatograph (Agilent) equipped with a flame ionization detector (FID), a splitless injector kept at 200 °C and an Optima 5-MS capillary column (length: 30 m; diameter: 0.25 mm; film thickness: 0.25 µm). Helium was used as carrier gas (1 $ml.min^{-1}$). The temperature ramp was set as above. The effluent was split into two deactivated fused silica capillary columns (100 cm x 0.25 mm), one leading 10 % of the outlet flow to an FID set à 300 °C and a second one leading 90 % of the outlet flow into a heated EAD transfer line kept at 200°C (Syntech Ockenfels). Antennal electrical responses to volatile compounds were tested and digitized using an IDAC board (acquisition controller IDAC-2; Syntech Ockenfels). Data was processed with a PC-based interface and software package

(GcEad 1.2.5, Syntech Ockenfels). Electrophysiological measurements were conducted on 8-12 females per cultivar. A compound was considered detected by *D. radicum* females when it elicited a clear depolarization response in at least half of the females tested.

Behavioral experiments

The effect of the VOCs detected by the females' antennae on their behavior was assessed using a dual-choice setup with artificial leaves. Leaves were made of a 10 cm green-paper straw (Utopia®) on which a green-paper limb (Cultura®) was stapled at 6 cm from the straw bottom. To reproduce a plant waxy cuticle, which is mandatory for *D. radicum* to lay eggs (Roessing and Städler, 1990) and thus to induce an exploration behavior, each leaf was briefly immersed in a 1 l glass beaker containing 800 ml of water and 40 g of paraffin wax, and heated at 85 °C in a water bath. Leaves were then placed individually in a Petri dish containing a 7 mm layer of river sand. Two leaves were placed in a 30 cm cubic nylon cage (BugDorm®, Taichung, Taiwan). A 2 x 2 cm filter paper was placed on a wooden rod inserted in the paper straw of each leaf, so that the filter paper overlaid the artificial leaf without touching it. On one filter paper, 10 µl of mineral oil containing a single VOC or a VOC mixture (each VOC at 1µg/µl) was deposited, whereas the second filter paper with only 10 µl of mineral oil served as control. VOCs were let impregnating the filter papers for 2 min before they were placed in the cage, then one female was released and the experiment started. Every 5 min during 90 min, it was recorded (i) whether the female was on a leaf and (ii) whether it was showing an exploration behavior. As preliminary experiments showed that VOCs alone were not sufficient to induce oviposition in these experimental conditions, the number of eggs laid was not recorded. Seven experiments were conducted, one with each single VOC detected by the females antennae, and three with VOC mixtures characterizing the three plant species studied (hereafter called '*B. oleracea* like', '*S. alba* like' and '*B. rapa* like'). All experiments took place at 20 ± 2 °C, 16L:8D, and 60 ± 10% RH, and 10-15 replicates were performed per experiment.

Statistical analyses

All analyses were performed using the R software version 3.5.0 (R Core Team 2018). Volatile profiles were compared multivariately using a redundancy analysis (RDA) on quadratic-root transformed and autoscaled data (Legendre and Legendre, 2012), with species and cultivar as independent factors. The effect of these factors was tested using a permutation F test with 9999 permutations (R package 'vegan'; Oksanen *et al.*, 2022). Pairwise comparisons between species and cultivars were performed also using permutation F-tests and p-values corrected with the False Discovery Rate method (Benjamini & Hochberg 1995). Analyses were based on the mean area of the extracted VOCs per plant. In each behavioral experiment, the probability of being present on a leaf or of showing an exploration behavior was compared between treatments (control *vs.* VOC or control *vs.* VOC mixture) using Wald tests applied on Generalized Linear Mixed Models (GLMM, distribution: binomial, link: logit), in which treatment, time and their interaction were considered as fixed factors and the cage as a random factor.

Estimated Marginal Means (EMMeans) were computed for interpretation (R package 'emmeans'; Lenth 2022). Three analyses were performed per experiment: one on the probability of being on the leaf (test of the attractiveness of the treatments at distance), one on the probability of being exploring the leaf when present (test of the ability of the treatments to stimulate an exploration behavior at contact) and one on the global probability of being exploring the leaf (integration of the two previous effects, i.e. test of the overall ability of the treatments to lead to an exploration behavior).

RESULTS

VOC emission profiles are characteristic of species but not cultivars

GC-MS analyses allowed the detection and identification of 12 different compounds, 10 being terpenes (eight monoterpenes and two sesquiterpenes) and the two others being ethylacetophenone and hexenyl acetate (Fig. 1a). Significant differences in volatile profiles were observed between species ($F = 12.66$, $p < 0.001$), and somehow between cultivars of *S. alba* (*S. alba*: $p = 0.065$, *B. rapa*: $p = 0.363$) (Fig. 1b). Each species was characterized by a specific profile. The specific signature of *B. oleracea* was made of α-farnesene and all monoterpenes. The specific signature of *S. alba* was mostly made of α-farnesene and hexenyl acetate (with all VOCs but hexenyl acetate emitted in greater amounts in cv. Sarah). The specific signature of *B. rapa* was mostly made of β-myrcene, β-ocimene, β-caryophyllene and ethylacetophenone (with all VOCs except β-caryophyllene emitted in greater amounts in cv. Richi).

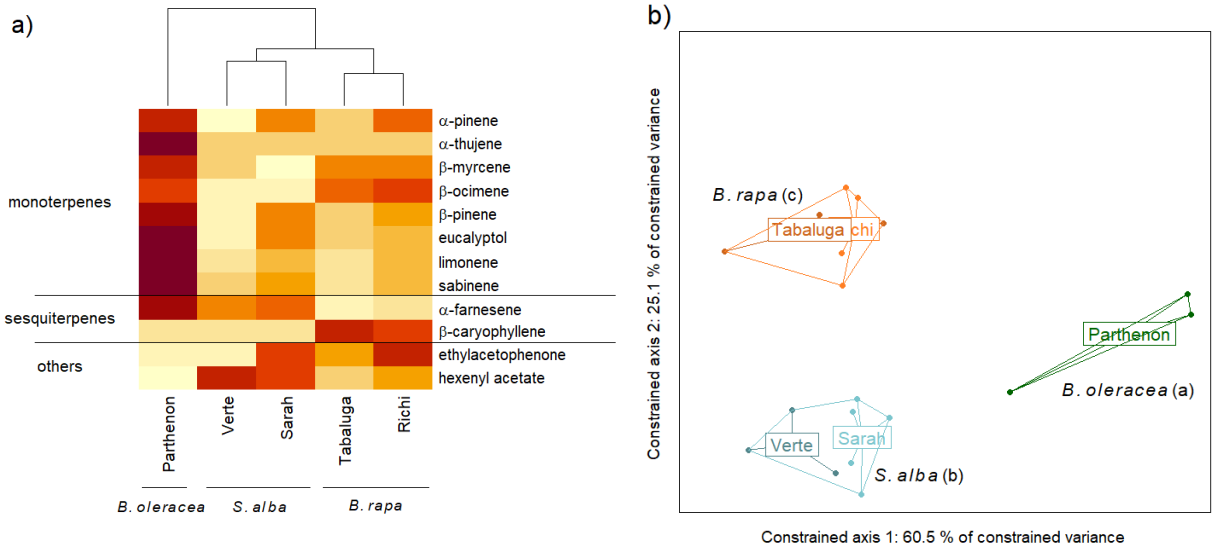

Fig. 1 (a) Heatmap of VOCs emitted from five cultivars of three Brassicaceae species (from mean values per cultivar). The emission level of each compound is compared between cultivars and represented by a color gradient from light yellow (lowest level) to dark red (highest level). (b) Score plot of the Redundancy Analysis (RDA) performed on the VOCs emitted from individual plants (orange: *Brassica*

rapa; blue: *Sinapis alba*; green: *B. oleracea*). Different bracketed letters indicate significant differences between species.

Four compounds among those present are detected by *D. radicum* females

GC-EAD analyses revealed that females responded to four compounds present in the VOC blends of the different cultivars: two monoterpenes (β-ocimene and sabinene) and two sesquiterpenes (α-farnesene and β-caryophyllene) (Fig. 2, 3a). The response to these compounds was confirmed with synthetic compounds (Menacer *et al.*, in prep.). Four females also responded to β-myrcene but only with VOC blends from cv. Tabaluga. As this compound is detected more intensely with an increasing dose (Menacer *et al.*, in prep.) and was more present in Parthenon than in Tabaluga (Fig. 1a), it was therefore removed from the following analyses since its detection was considered inconsistent and unreliable. A statistical analysis based on the detected compounds only, still highlighted interspecific differences in volatile profiles ($F = 14.77$, $p < 0.001$), but none at the intraspecific scale (*S. alba*: $p = 0.103$, *B. rapa*: $p = 0.338$) (Fig. 3b). A species-specific signature was shown again (Fig. 3a), with a blend of β-ocimene, sabinene and α-farnesene for *B. oleraceae* (Fig. 2a), sabinene and α-farnesene for *S. alba* (Fig. 2b) and β-ocimene, sabinene and β-caryophyllene for *B. rapa* (Fig. 2c).

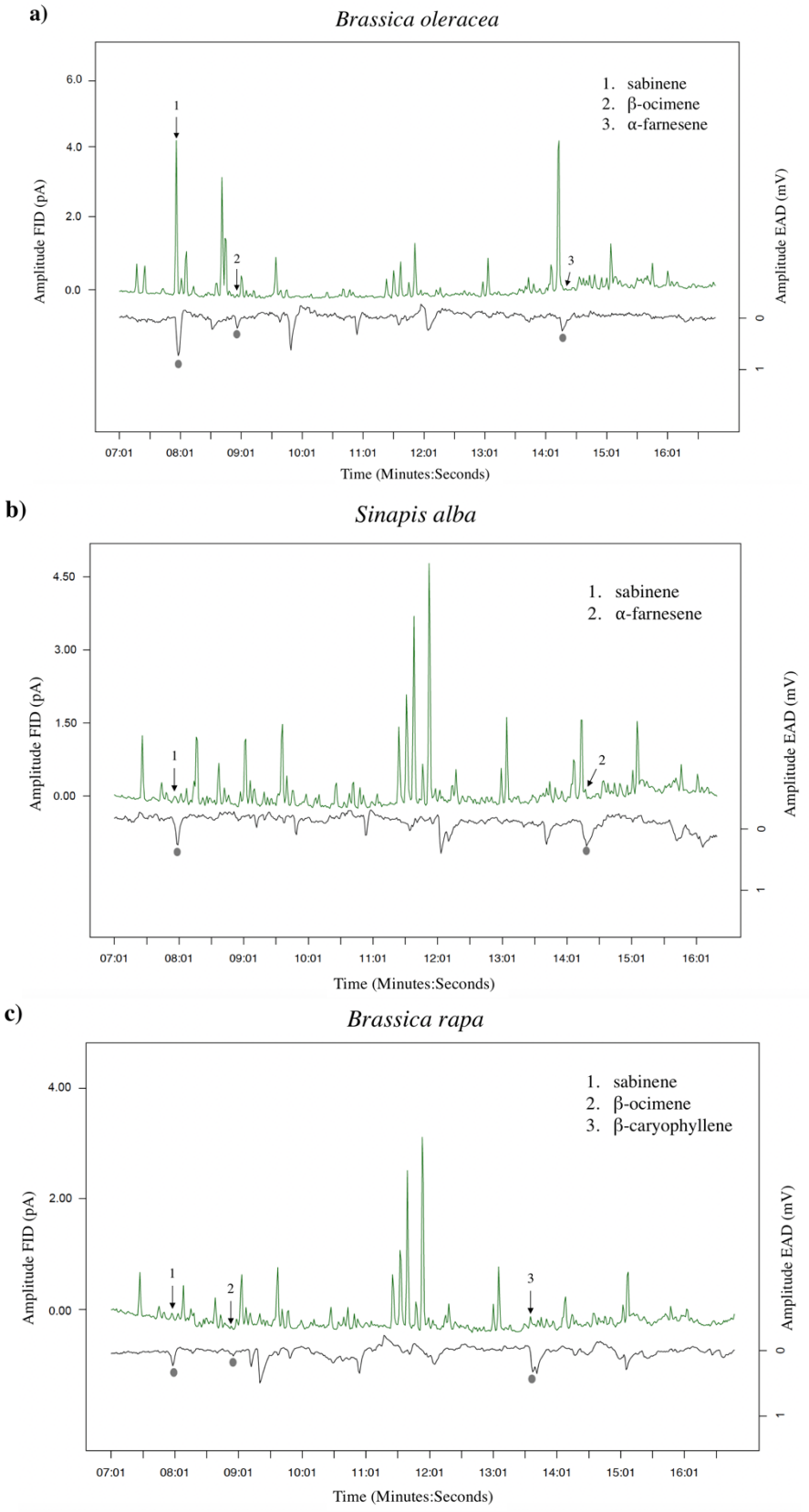

Fig. 2 Examples of antennal responses of *Delia radicum* females to VOCs from headspaces of a) *Brassica oleracea* (cv. Parthenon), b) *Sinapis alba* (cv. Sarah) and c) *B. rapa* (cv. Richi). Grey circles represent the antennal responses of females to the different compounds identified

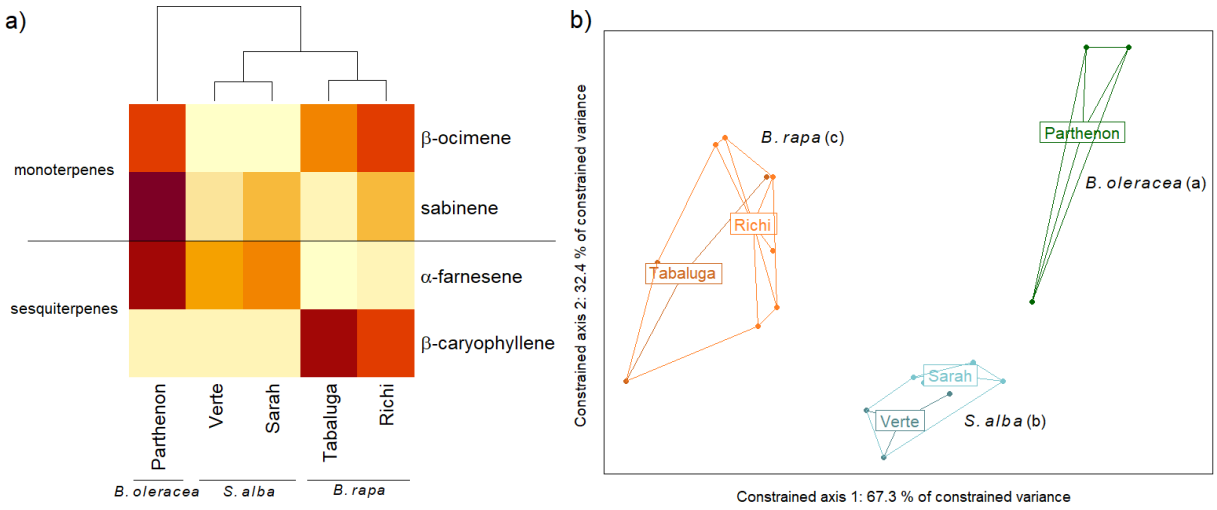

Fig. 3 (a) Heatmap of VOCs detected by female's *Delia radicum* antennae and emitted from five cultivars of three Brassicaceae species. The emission level of each compound is compared between cultivars and represented by a color gradient from light yellow (lowest level) to dark red (highest level). (b) Score plot 1-2 of the Redundancy Analysis (RDA) performed on the VOCs compounds emitted from five cultivars of three Brassicaceae species and detected by female's *Delia radicum* antennae (orange: *Brassica rapa*; blue: *Sinapis alba*; green: *B. oleracea*). Different bracketed letters indicate significant differences between species.

VOCs have biological effects mostly when combined, with effects being either at distance or at contact

Following results of the GC-EAD recordings, four COVs were retained for behavioral experiments (β-ocimene, sabinene, α-farnesene and β-caryophyllene) as well as the mixtures β-ocimene + sabinene + α-farnesene ('*B. oleracea* like'), sabinene + α-farnesene ('*S. alba* like') and β-ocimene + sabinene + β-caryophyllene ('*B. rapa* like'). When tested alone, one of the four VOCs (sabinene) did not induce neither attraction nor stimulation at contact and two (α-farnesene, β-ocimene) seemed to induce repulsion (Fig. 4a). None of these three compounds induced exploration when considered globally (Fig. 4c). Only β-caryophyllene induced attraction, but not stimulation at contact. Overall, β-caryophyllene led to increase the probability to show an exploration behavior, but this probability was very low (Fig. 4c). Contrary to most single VOCs, all VOC mixtures increased the global probability to show an exploration behavior, significantly or nearly (Fig. 4b and 4c). However, VOC mixture had contrasted effects: the '*B. rapa* like' mixture showed significant attraction but not stimulation at contact, the '*B. oleracea* like' had opposite effect and the '*S. alba* like' showed significant repulsion but a high stimulation at contact (Fig. 4).

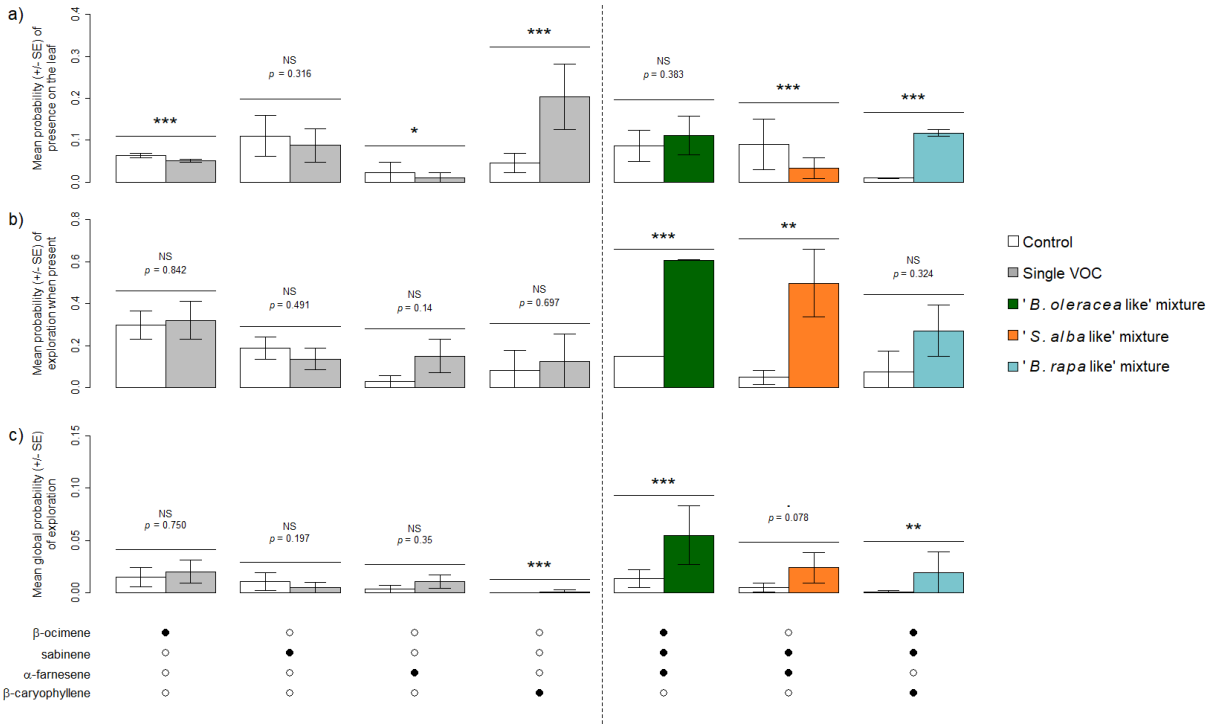

Fig. 4 Behavioral response of *Delia radicum* females to the VOCs emitted by three Brassicaceae species and eliciting an antennal response, when tested alone or in mixtures. (a) Mean probability (± SE) of being present on artificial leaves (distance effect). (b) Mean probability (± SE) of showing an exploration behavior when present (contact effect). (c) Mean probability (± SE) of showing an exploration behavior (global effect). Black dots indicate the presence of the VOC in the treatment tested. NS: $p > 0.1$, . : $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. N = 10-15 per experiment.

DISCUSSION

Plant-emitted VOCs are well known to mediate distance attraction of phytophagous insects (Bruce *et al.*, 2005). However, much less studied is the fact that VOCs can also act on insects' behavior once in contact with the plant (Gouinguené *et al.*, 2005, Braccini *et al.*, 2015). Moreover, when the effect of VOCs on the host selection process is investigated, the distinction between distance and contact effects is only rarely made. The present study highlights the importance of making this distinction by showing that pre- and post-contact effects of VOCs can be independent.

Olfactometer experiments had already shown that *D. radicum* females are attracted differently to the three plant species tested here (Kergunteuil *et al.*, 2015a, 2015b). Using GC-MS identification and subsequent multivariate analyses, we show that these species could be distinguished based on their VOC blends, and each characterized by a specific emission profile. These results are in agreement with the literature since species-specific blends are usually observed (*e.g.* Schoonhoven *et al.*, 2005; Van Den Boom *et al.*, 2004; Bukovinszky *et al.*, 2005; Zouaoui *et al.*, 2020). The specificity of the blends

characterized in our study has already been reported (Kergunteuil *et al.*, 2015a), and although species-specific mixtures identified here are not exactly similar to those described previously, all VOCs identified here but the aromatic ethylacetophenone have already been shown to be emitted by these species (Tollsten and Bergström 1988; Kergunteuil *et al.*, 2015a). The specificity of the mixtures emitted by each plant species appears logically as a starting point for phytophagous insects to evolve interspecific discrimination abilities.

Electrophysiological recordings revealed that not the whole blends are detected by females' antennae, but only four ubiquitous terpenes which combinations are species-specific. While a blend of plant VOCs may contain more than a hundred compounds, electrophysiological recordings have shown that only a subset of these compounds is usually detected by insects (Bruce *et al.*, 2005), which is confirmed by our results. Among the electrophysiologically active compounds, behavioral experiments showed that β-caryophyllene had an attractive effect on *D. radicum* females, which supports an important role of this compound in plant-insect interactions as it has been shown attractive to a diversity of generalist and specialist phytophagous insects (*e.g.* Cha *et al.*, 2008, 2011; Sadeh *et al.*, 2017; Zhang, 2018; Hosseini *et al.*, 2021). However, it is the only VOC which induced attraction or exploration when applied alone, while all three VOC mixtures had such effect. Indeed, β-ocimene, α-farnesene and sabinene appeared neutral or even repellent alone, whereas their combinations increased the probability to observe an exploration behavior. Consistently, although the behavioral pattern was quite similar between β-caryophyllene alone and the only mixture that included it, the global probability of exploring the leaf appeared higher when β-caryophyllene was combined with β-ocimene and sabinene. These results are in line whit the many experiments showing that stronger behavioral responses are obtained with specific blends or combinations of VOCs rather than with individual compounds (Bruce and Pickett, 2011). Furthermore, the striking example of Webster *et al.* (2008a, 2008b, 2010) clearly demonstrates that the signaling value of a mixture of VOCs is determined by the whole mixture rather than by the sum of the signaling values of its individual components. Although not all possible combinations were tested in our study, our results clearly support this idea. Nonetheless, further behavioral studies with more realistic concentrations and ratios of VOCs would be necessary to conclude about natural host selection processes in *D. radicum*, as these two factors are known to be of importance (Webster *et al.*, 2008; Bruce and Pickett, 2011; Cha *et al.*, 2011).

VOCs have long been recognized as key cues mediating distance attraction in phytophagous insects (e.g. Cornelius *et al.*, 2000, Padmaja *et al.*, 2010; Biasazin *et al.*, 2014). Their involvement in oviposition stimulation once in contact with the plant has also been shown in several species, although much less studied (Proffit *et al.*, 2011; Kamala Jayanthi *et al.*, 2014; Binyameen *et al.*, 2021, Roh *et al.*, 2021). Our behavioral experiments confirm that in *D. radicum*, VOCs induce pre-ovipositional exploration behaviors at contact. Moreover, although the global probability of *D. radicum* to explore artificial leaves, which results from both the distance attractiveness and the ability to stimulate exploration behaviors

once in contact, was significantly (or nearly) increased by all three VOC mixtures, the effect of these mixtures was strikingly different: for the *'B. rapa* like' mixture this effect is due to an increased attractiveness, while for the '*S. alba* like' and '*B. oleracea* like' mixtures it is due to a higher stimulation of exploration behaviors once in contact with the leaf. This demonstrates the relevance of distinguishing between pre- and post-contact phases when studying the effect of VOCs on host selection processes. Indeed, it shows that a given behavior can be initiated by different and independent mechanisms, although these are mediated by the same kind of plant cues.

In conclusion, this study provides a better understanding of the action mechanism of plant-emitted VOCs on the behavior of a specialist phytophagous insect, and confirms the importance of VOC combinations in mediating plant-insect interactions. More importantly, it demonstrates that the effects of these chemical cues may be independent when considered at distance and once the insect makes contact with the plant, and that a given type of cues (here, VOCs) can mediate several aspects of host recognition processes of phytophagous insects.

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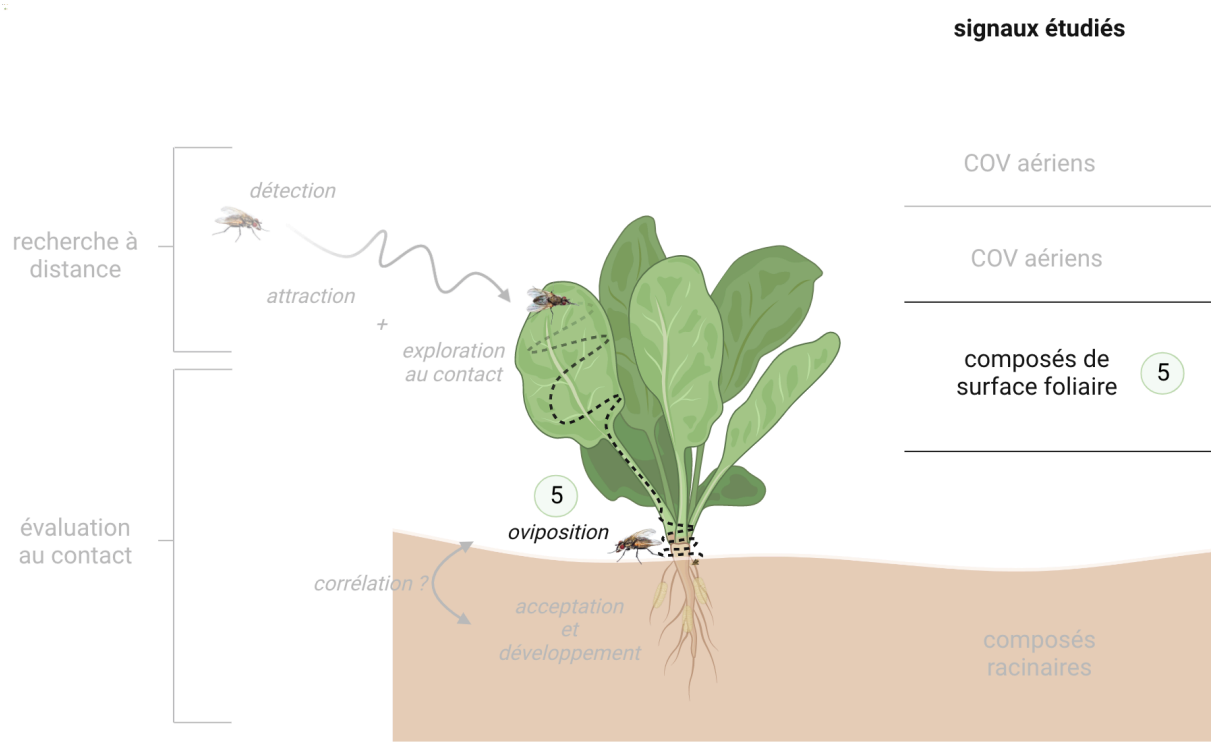
Article 5

Host recognition mechanisms can vary within an insect herbivore's host range: oviposition is not always triggered by leaf surface compound



Host recognition mechanisms can vary within an insect herbivore's host range: oviposition is not always triggered by leaf surface compounds

Manuscript ready for submission in Oecologia

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ABSTRACT

Host plant selection is a key step in the life cycle of phytophagous insects and involves a wide variety of plant cues. Contact chemical cues, particularly leaf surface compounds, often determine the final acceptance of the plant. However, studies investigating the ecological role of surface compounds are usually assessed either at the intraspecific or interspecific scale, rarely at both, which may not allow to draw general conclusions about the determinants of oviposition behaviors, especially regarding host discrimination. Cabbage root fly (*Delia radicum*) females exhibit oviposition preferences both between and within host plant species, suggesting fine discrimination abilities. The present study aimed to test whether leaf surface compounds are the cues allowing such discrimination, at both the inter- and intraspecific scale. Three host species were compared, namely *Brassica rapa*, *B. oleracea* and *Sinapis alba*, as well as two populations (here, cultivars) of *B. rapa* and *S. alba*. These species and populations have previously been shown contrasted in the stimulation of oviposition. Surface compounds were extracted in a solvent mixture and their effect assessed using an artificial leaf device. Our results confirm that for *B. rapa* and *B. oleracea*, surface compounds mediate oviposition stimulation, unlike those of *S. alba* which are not stimulant at all although this species is a true host plant. This study shows that multiple different cues may be involved in host selection behaviors, and thus challenges the idea of a single host recognition mechanism in a given insect species. It reminds the importance of testing multiple plant species before drawing general conclusions about host recognition mechanisms in phytophagous insects.

Keywords: leaf surface compounds; oviposition stimulation; host plant selection; *Delia radicum*

INTRODUCTION

In phytophagous insects, the host selection process implies several phases involving a large diversity of plant cues. Once the insect has located a plant using visual (Harris and Miller 1983; Schoonhoven *et al.*, 2005) and olfactory (Bruce *et al.*, 2005) cues, the leaf surface is usually the initial contact area. In many cases, females do not sample the internal content of the leaves prior to egg laying, and thus use only chemical cues present on the plant surface, i.e. on or in epicuticular waxes. In addition to providing information on the phenological stage, physiological state or prior interactions with other organisms (Städler *et al.*, 2002; Chapman, 2003; Mamrutha *et al.*, 2017; Tomasi *et al.*, 2018), epicuticular waxes inform about the plant identity. Indeed, the quality and quantity of epicuticular waxes can vary greatly between different species, as well as between different genotypes of the same species (Griffiths *et al.*, 2001; Mobarak *et al.*, 2020, Debnath *et al.*, 2021). These differences in chemical composition may allow insects to recognize their host plant and choose a particular site for oviposition (Müller and Riederer, 2005). Despite numerous studies showing the involvement of surface compounds in females' oviposition site recognition (Riederer and Müller, 2006), relatively few of them have investigated the involvement of such compounds in oviposition preferences between different host plants. Moreover, studies that have done so have either focused on their influence on preferences at the intraspecific level (*e.g.* Juvik *et al.*, 1988; Derridj *et al.*, 1996; Cervantes *et al.*, 2002) or at the interspecific level (*e.g.* Foster and Harris, 1992; Fernández *et al.*, 2019), rarely at both scales (but see Kanno and Harris, 2000). However, quantitative differences (i.e. similar compounds but in different concentrations) are mainly expected within the same species whereas qualitative differences (i.e. different compounds) are mainly expected between different species. Therefore, comparisons between these two scales should allow drawing more general conclusions about the contribution of surface compounds to the host selection process.

Adult *Delia radicum* live aboveground and females are specialists of Brassicaceae for oviposition (Capinera, 2008). Host selection by females is partly determined by plant physical cues such as color, shape or texture (Prokopy *et al.*, 1983; Roessingh and Städler 1990; Košťál 1993). However, the most important cues for host recognition and selection are plant chemical compounds. During the contact phase with the plant, females show a stereotypical behavior of host exploration (Zohren, 1968): they walk on the surface of the leaf in a circular movement and then move down the stem and circle the plant collar several times before depositing their eggs, or not, depending on the cues perceived (Finch and Collier, 2000). This behavior suggests that surface compounds play a key role in stimulating oviposition in *D. radicum*, which has been confirmed in a number of studies (Städer and Schöni, 1990; Roessingh *et al.*, 1992; Kostal, 1993; Baur *et al.*, 1996; Hurter *et al.*, 1999; de Jong *et al.*, 2000; Marazzi and Städler, 2004; Griffiths *et al.*, 2001). However, almost all of these studies focused on one plant species or populations only, while *D. radicum* females exhibit oviposition preferences both between species and among different populations of the same species (Doane and Chapman 1962; Radcliffe and Chapman

1966; Ellis *et al.*, 1999, Lamy *et al.*, 2020; Menacer *et al.*, 2021). To our knowledge, only Baur *et al.* (1996) has focused both on several species and several genotypes of the same species. In this study, two brassicaceous species and two populations per species were tested, and oviposition preferences of *D. radicum* females on whole plants correlated positively with those on artificial leaves with surface extracts, both intra- and interspecifically. However, how general is the involvement of surface compounds in the host plant selection process of this species remains unknown.

Recent studies showed oviposition choice contrasts between three host species: Chinese cabbage (*Brassica rapa* ssp. *Pekinensis*), cabbage (*B. oleracea*), and white mustard (*Sinapis alba*), with a clear preference for *B. rapa*, then *S. alba* then *B. oleracea* (Menacer *et al.*, 2021; Lamy *et al.*, 2020). They also showed intraspecific preferences within *B. rapa* and *S. alba*. The present study aimed at assessing the importance of specific surface cues in these preferences, by extracting plant surface compounds and testing their effect on the oviposition intensity using an artificial leaf device inspired from Städler and Schöni (1990).

MATERIALS AND METHODS

Insects and plants

Females of *D. radicum* originated from a laboratory rearing initially constituted from a field population collected in the field in 2019 (Pleumeur-Gautier, Brittany, France, 48°48′11″N, 3°09′21″W). Flies were reared on rutabagas as described in Neveu Bernard-Griffiths (1998) and fed with a milk powder: yeast: sugar (1:1:1) mixture. Adults were identified based on sexual dimorphism in the size of the eye-spacing, which is much more pronounced in females than in males (Smith 1927). Females used in the experiments were 7–10 days old and mated with no prior oviposition experience. Five commercial cultivars were used in all experiments: two of *Brassica rapa* ssp. *pekinensis* ('Richi' and 'Tabaluga'), two of *Sinapis alba* ('Sarah' and 'Verte') and one of *B. oleracea* ('Parthenon'). Single seeds were sown in individual 3.5 × 3.5 × 5 cm clumps filled with potting soil (Premier Tech Horticulture), and plants were grown in a climatic chamber at optimum conditions (20 ± 1 °C, 16L:8D and 70 ± 10% RH) where they were watered twice a week with a nutrient solution (Hoagland and Arnon, 1950). All plants were used at the 3-4 true leaves stage.

Leaf surface compounds extraction

Leaf surface compounds were extracted by cutting leaves at the petiole and dipping the limb for 30 sec into a glass beaker under agitation (130 rpm) containing 15 ml of a mixture of hexane, dichloromethane and methanol 80:10:10, allowing the extraction of apolar, moderately polar and polar compounds respectively. Ten samples of 4-7 plants each were constituted per cultivar, 3-4 leaves being extracted per plant. Before extraction, a picture of each leaf was taken under standardized light conditions and

distance, and its surface was determined using ImageJ®. After extraction, the supernatant was collected and transferred into 20 ml glass tubes. Solvents were then completely evaporated under nitrogen flow and resuspended into 10-20 ml of the same solvent mixture, the solvent volume depending on the initial leaf surface extracted.

Oviposition tests with artificial leaves

Artificial leaves used in the experiments were made of 10 cm green paper straws (Utopia) on which green paper limbs (Cultura) were stapled at 6 cm from the bottom. To reproduce a plant waxy cuticle, which is crucial for *D. radicum* oviposition (Roessingh and Städler, 1990), each leaf was entirely dipped into a 1 l glass beaker containing 800 ml of water and 40 g of paraffin wax, and heated in a water bath at 85 °C. Once the paraffin wax dried, 600 µl of surface extract was deposited using a precision sprayer (Lenz®), divided into six sprays of 100 µl each. Two sprays on the leaf limb and one on the stem were performed per leaf side to cover the entire setup. Leaves were then placed individually in a Petri dish containing a 7 mm layer of moistened river sand and the solvent was left to evaporate for 15 min before starting the experiments.

Female oviposition was investigated using a multi-choice set-up composed of a 30 cm cubic nylon cage (BugDorm, Taichung, Taiwan), which contained three or six artificial leaves randomly arranged in a circle and a water dispenser made from cotton at its center. Two types of experiments were conducted: an interspecific test between the five cultivars of the three species and intraspecific tests between either *B. rapa* or *S. alba* cultivars. In all experiments, one artificial leaf covered with surface extract from each cultivar tested was placed in the cage and one artificial leaf covered with the pure solvent mixture was added as control. Six females were released in cages for the intraspecific tests and 12 females for the interspecific ones (thereby keeping the same ratio of two females per artificial leaf in both experiments). After 24 h, the total number of eggs laid per artificial leaf was counted by separating these eggs from the sand by flotation. All experiments took place in a climatic room at 20 ± 2 °C, 16L:8D, and 60 ± 10% RH and fifteen cages were set-up per experiment.

Statistical analyses

All analyses were performed using the R software version 4.1.3 (R Core Team 2022). In all experiments, the proportions of eggs laid per cultivar were compared using Wald tests applied on multinomial models (R package 'RVAideMemoire'; Hervé 2022).

RESULTS

Interspecific preference test

A significant preference was observed when females were given the choice between leaves covered with surface extracts from cultivars of the three species. At the interspecific scale, all species were different from each other with *B. rapa* being the most preferred species, then *B. oleracea* and *S. alba* the least with no significant difference observed between *S. alba* and control leaves (Fig. 1). An intraspecific difference was also observed between the two cultivars of *B. rapa* with a preference for cv. Tabaluga, which received a proportion of eggs nearly 1.4 times higher than the cv Richi. An intraspecific preference was also observed within *S. alba* with a preference for cv. Sarah, but very few eggs were laid on both cultivars (overall mean ± SE 0.54 ± 0.26).

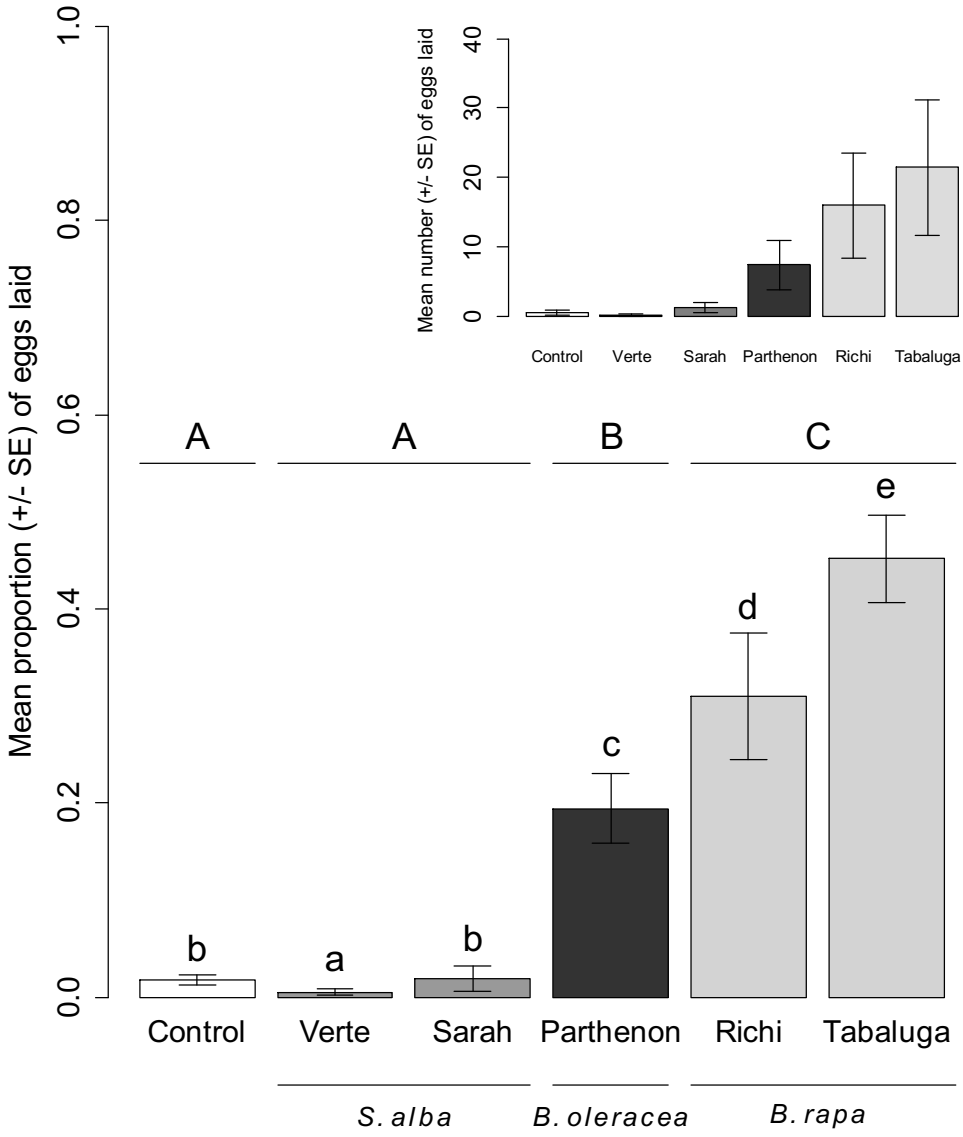

Fig. 1 Mean proportion (± SE) of eggs laid per artificial leaf coated with leaf extracts from different plant species and cultivars by *Delia radicum* females. The insert represents the mean number (± SE) of

eggs laid per artificial leaf. Different uppercase letters indicate significant differences between species, whereas different lowercase letters indicate significant differences between cultivars. N = 15 cages.

Intraspecific preference test

When given the choice between artificial leaves covered with surface extracts of only two different cultivars of either *B. rapa* or *S. alba*, females showed significant intraspecific oviposition preferences but only in *B. rapa*. Similarly to the interspecific experiment, a preference for cv. Tabaluga was observed with nearly 1.4 times more eggs laid than on cv. Richi and over seven times more eggs than on the control leaf (Fig. 2a). In addition, leaf surface extracts of *B. rapa* were highly stimulant: the mean number of eggs laid on both *B. rapa* cultivars was almost eight times higher than the number of eggs found on control leaves (Fig. 2a). In contrast and in line with observations of the interspecific experiment, almost no egg was laid when leaf extracts of *S. alba* were proposed to females (Fig. 2b). Contrary to the interspecific preference experiment, no difference in the proportion of eggs laid was observed between the two cultivars (Fig. 2b).

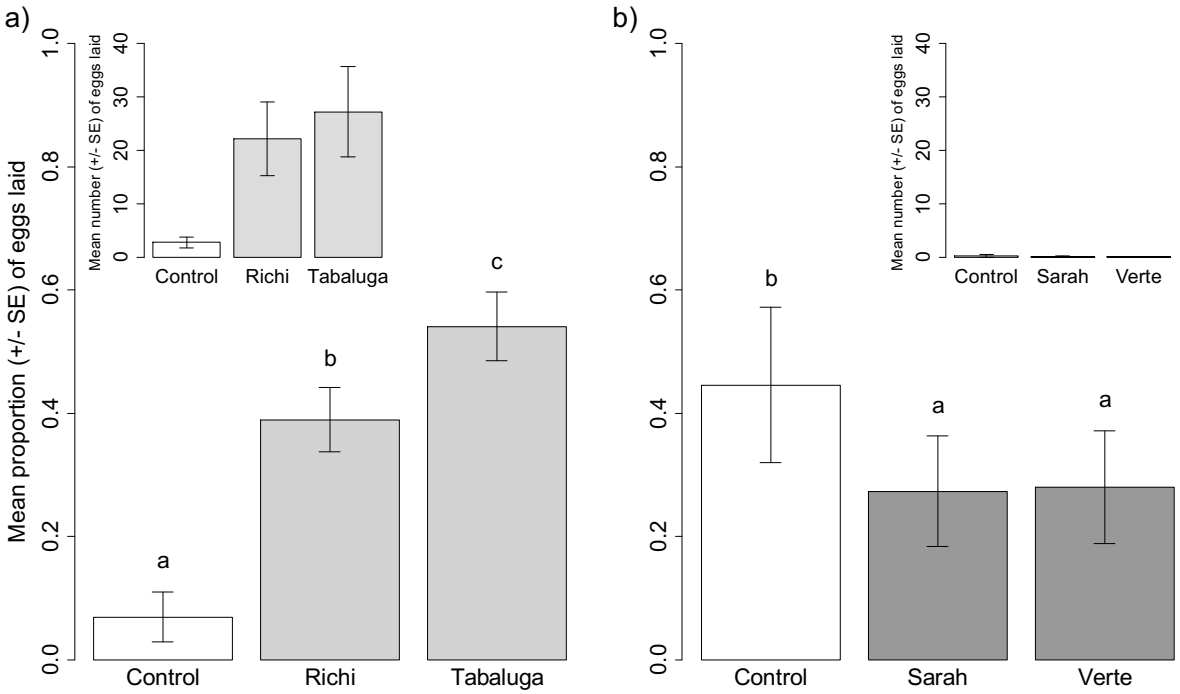

Fig. 2 Mean proportion (± SE) of eggs laid by *D. radicum* per artificial leaf coated with leaf extracts from different cultivars of (a) *Brassica rapa* and (b) *Sinapis alba*. The inserts represent the mean number (± SE) of eggs laid per artificial leaf. Different letters indicate significant differences between treatments. N = 15 cages per experiment.

DISCUSSION

A number of studies have shown that *D. radicum* females have oviposition preferences within their host range, both between and within plant species (Doane and Chapman 1962; Radcliffe and Chapman 1966, Lamy *et al.*, 2020; Menacer *et al.*, 2021). Baur *et al.* (1996) suggested that leaf surface compounds may be responsible for these preferences, a hypothesis that the present study tested. If confirmed in some cases, our results show that this is not a general rule and that oviposition may be triggered by different cues depending on the plant species.

At the interspecific scale, leaf surface compounds of *B. rapa* highly stimulated oviposition while those from *B. oleracea* were slightly less stimulating, which is consistent with preferences observed in previous experiments on whole plants (Lamy *et al.*, 2020; Menacer *et al.*, 2021). Moreover, a preference between the two cultivars of *B. rapa* was observed and confirmed in the intraspecific experiment. Despite inconsistencies with whole-plant observations for the intraspecific contrast in *B. rapa*, where cv. Richi was preferred over cv. Tabaluga (Lamy *et al.*, 2020; Menacer *et al.*, 2021), these results suggest that females are able to detect species- and population-specific surface cues which are involved in host recognition and in oviposition preferences. Since specialists must be able to identify their host plants in a non-host environment, many have evolved to use unique specialized metabolites of their host plants as cues for host finding and identification (Macel & Vrieling, 2003; Nieminen *et al.*, 2003). In particular, brassicaceous plants are known to exude specialized metabolites on their leaf surface, such as glucosinolates (Mithen, 2001; Fahey *et al.*, 2001; Wiesner *et al.*, 2013) and thia-triaza-fluorene compounds (Roessingh *et al.*, 1997; Hurter *et al.*, 1999). These are mostly deterrent for generalist herbivores but are used as cues for host recognition and acceptance by many specialists (Miller and Strickler, 1984; Wittstock *et al.*, 2003; Hopkins *et al.*, 2009), including *D. radicum* (Nair *et al.*, 1976; Städler and Schöni, 1990; de Jong *et al.*, 2000; Marazzi *et al.*, 2004). Variations in such specialized metabolites on the leaf surface have been described both between and within species (Griffiths *et al.*, 2001, Hopkins *et al.*, 1997). This suggests that the presence of one or a combination of these compounds might be characteristic of the species and cultivars tested here, and may explain preferences we observed, as shown by Baur *et al.* (1996) in the discrimination among resistant and susceptible Brassica genotypes. Further chemical analyses of leaf surface extracts would be necessary to confirm this hypothesis.

Strikingly, almost no egg was laid when leaf surface extracts of *S. alba* were sprayed on artificial leaves, which also contradicts whole-plant observations from the laboratory (Menacer *et al.*, 2021) and the field (Kergunteuil *et al.*, 2015a) where this plant was found to stimulate oviposition. This absence of stimulation of *S. alba* extracts was observed in the interspecific experiment, where *B. rapa* extracts were very stimulating and could hide minor effects, but also in the intraspecific experiments where only *S. alba* extracts were present. This shows that in this species, surface compounds are not involved in the

stimulation of oviposition, at least not alone. Many studies have shown the involvement of surface compounds in the stimulation or deterrence of oviposition in phytophagous insects (Riederer and Müller, 2006), with an absence of effect being an exception (*e.g.* Howlett and Clarke, 2003). However, the large majority of these studies have been conducted on a single plant species, or different populations of the same species, but only rarely at both scales (Kanno and Harris, 2000). Here, we demonstrate that when confirmed, the involvement of surface compounds in oviposition stimulation may not be generalizable to all plant species of a given insect's host range. These results provide additional crucial information to the hypothesis of Baur *et al.* (1996), by suggesting that the nature of the stimuli leading to oviposition may be different depending on the plant species, promoting the idea that no conclusion on the host plant selection process could be made up based on a single plant species.

The differences between previous results on oviposition preferences with whole plants and our results with leaf surface extracts suggest that cues other than surface compounds are used by *D. radicum* females to discriminate their host plant. This would not be surprising, as surface compounds are not the only chemical cues involved in the host selection process in phytophagous insects, even at contact. In particular, although volatiles are mostly known to play a key role in host location at distance (Bruce *et al.*, 2005), once on a plant, females can also perceive certain volatiles, leaving the inner leaf tissues either through the cuticle or stomata (Müller and Riederer, 2005) or adsorbed and re-released from plants (Choh *et al.*, 2004; Himanen *et al.*, 2010). While host selection for oviposition is generally considered to be predominantly guided by surface compounds (Städler, 1984), volatile compounds may also be important by acting synergistically with contact compounds. For example, in many specialists of brassicaceous plants, volatile hydrolysis products of glucosinolates, especially isothiocyanates, have synergistic effects as attractants (Bartlet *et al.*, 1993; Mewis *et al.*, 2002) and oviposition stimulants (Städler *et al.*, 2002; Renwick *et al.*, 2006; Sun *et al.*, 2009). Furthermore, several studies reported that volatiles can also elicit an ovipositional response alone (Dweck *et al.*, 2013; Damodaram *et al.*, 2014; Asmare *et al.*, 2017; Hosseini *et al.*, 2021). In *D. radicum*, volatiles are already known to mediate attraction of females toward host plants (Finch, 1978; Nottingham, 1988; Finch and Collier, 2000; Kergunteuil *et al.*, 2012, 2015a, 2015b), and some volatiles can directly mediate their oviposition behavior (de Jong and Städler, 1999; Menacer *et al.*, in prep). However, further studies integrating volatiles emitted by leaves would be necessary to confirm the hypothesis of a synergy between volatile and contact chemical cues, especially in the case of *S. alba*.

Overall, this work contributes to the scarce literature on the involvement of plant surface compounds in inter- and intraspecific oviposition preferences in phytophagous insects, and suggests that multiple cues may be involved in these behaviors. Furthermore, it challenges the idea of a unique mechanism of host recognition in a given insect species, and underlines that a multiplicity of species or populations are necessary to draw more general conclusions about host plant selection processes in phytophagous insects.

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Discussion générale

Dans l'introduction de ce manuscrit, nous avons vu que la sélection de la plante hôte chez les insectes phytophages implique différentes phases comportementales, à la fois chez les adultes et chez les larves, qui sont en grande partie médiées par les signaux chimiques des plantes (Schoonhoven *et al.*, 2005). La nature des signaux à l'origine de la discrimination d'un hôte est largement décrite dans la littérature, contrairement à leur implication dans les préférences inter- et intraspécifique des insectes qui semble pourtant nécessaire pour comprendre les mécanismes qui sous-tendent la sélection de l'hôte. De plus, nous avons vu que cette sélection ne reposait pas uniquement sur de choix des femelles mais que les larves, aériennes comme souterraines pouvaient également être impliquées dans l'acceptation ou le rejet de la plante sur laquelle elles avaient été déposées (Schoonhoven, 1987 ; Johnson, 2013). L'objectif général de cette thèse était donc de progresser dans notre compréhension du processus de sélection de l'hôte en décryptant les mécanismes impliqués dans l'acceptation de l'hôte et les préférences inter- et intraspécifiques des larves et des adultes chez un insecte phytophage spécialiste. Cette discussion générale se compose de deux grandes parties qui vont respectivement traiter de la complexité du message utilisé par les femelles pour accepter et discriminer différents hôtes, et du lien entre le choix des femelles et celui de leurs larves, à travers une synthèse des résultats obtenus. Des résultats complémentaires d'expérimentations faites au cours de cette thèse ou dans des projets parallèles ainsi que différentes perspectives au travail présenté dans ce manuscrit seront également présentés afin d'enrichir la réflexion.

I. La complexité du message utilisé par les femelles pour la reconnaissance et l'acceptation de l'hôte

Lorsque des plantes hôtes de différentes espèces et cultivars ont été proposés aux femelles, nous avons pu mettre en évidence des contrastes d'oviposition, à la fois à l'échelle inter- et intraspécifique, qui traduisent des préférences dans le choix des femelles au sein de leur gamme d'hôtes (**article 1**). Si les femelles montrent une préférence pour certaines espèces ou certains cultivars, cela signifie qu'elles sont capables d'établir une hiérarchie entre les différentes plantes hôtes basée sur la reconnaissance et la discrimination des signaux spécifiques qu'elles perçoivent. Nous avons vu dans l'introduction que la spécificité de ces signaux résidait en grande partie sur la diversité des profils chimiques à la fois des composés de contact et volatils. Nous nous sommes donc focalisés sur ces deux signaux pour tenter de comprendre comment ils étaient impliqués dans la reconnaissance et la discrimination des différents hôtes par les femelles.

1. Le signal volatil

Pour étudier l'implication des COV dans la discrimination des différents hôtes chez les insectes phytophages, la première étape classique est d'effectuer des tests comportementaux à l'aide d'un dispositif d'olfactométrie afin de déterminer l'attractivité relative des différentes plantes pour l'insecte. Au cours de ce travail de thèse, un dispositif d'olfactométrie en tube droit a été mis en place (Fig. 1) afin d'évaluer l'attractivité des trois espèces utilisées dans l'**article 1**, ainsi que deux cultivars de *Brassica rapa* et *Sinapis alba* identifiés comme contrastés dans les préférences des femelles. Les résultats de ces expériences semblaient indiquer une attraction des femelles par les deux cultivars de *S. alba* (Fig. 2), ce qui aurait permis d'envisager une reconnaissance de cette espèce de plante uniquement à partir des COV qu'elle émet. Cependant, ces résultats n'étaient pas du tout en accord, ni avec ceux de Kergunteuil *et al.* (2015b) qui avaient montré avec le même type d'olfactomètre un contraste d'attractivité entre les différentes espèces (*B. rapa* > *B. oleracea* > *S. alba*), ni avec toute la littérature qui montre que le signal volatil émis par *B. oleracea* ou *B. rapa* (et d'autres plantes du genre *Brassica*) est attractif pour les femelles (Traynier, 1967b ; Finch, 1978 ; Hawkes et Coaker, 1979 ; Finch et Skinner, 1982a ; Kergunteuil *et al.*, 2015a, 2015b). Le doute quant à la fiabilité des observations effectuées au cours de ces tests nous a conduits à ne pas exploiter ces résultats dans le cadre des différents articles de ce manuscrit.

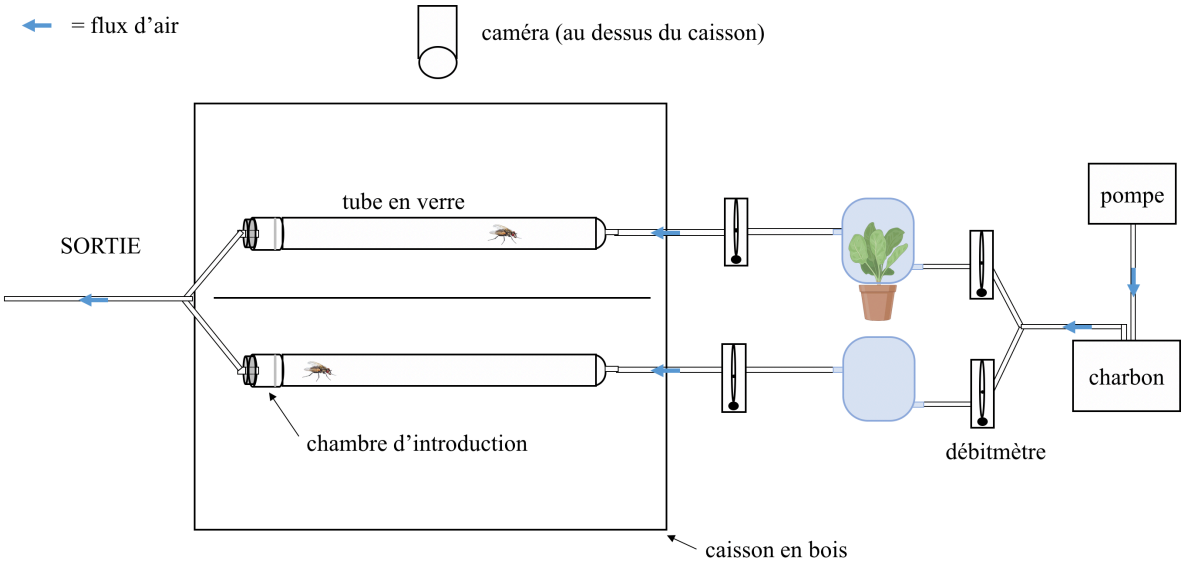

Figure 1 : Schéma du dispositif de non-choix d'olfactométrie en tube droit. Les observations sont filmées dans un caisson à l'éclairage homogène et analysées ensuite sous Ethovision® (Noldus). Chaque session d'observation (10 minutes) compare systématiquement le comportement de 2 femelles : une qui est exposée aux COV d'une plante et l'autre à de l'air pur.

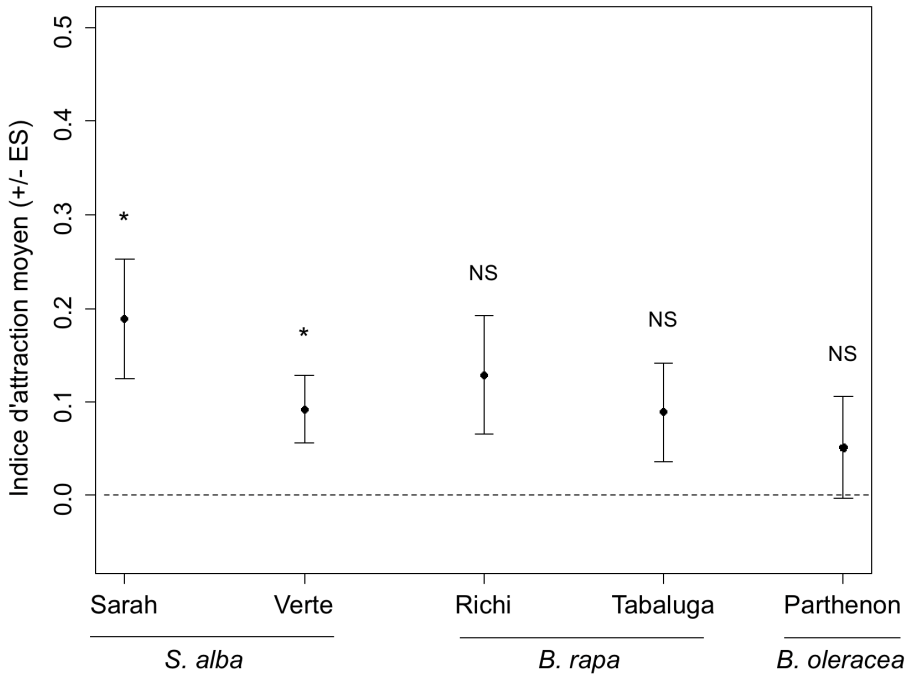

Figure 2 : Indice d'attraction moyen (± SE) des femelles *D. radicum* pour les différents cultivars testés dans un dispositif d'olfactométrie en tube droit. Les tubes ont été divisés artificiellement en six sections de 10 cm chacune et le temps passé dans chaque section a été calculé à l'aide du logiciel de capture vidéo en temps réel Ethovision® (Noldus). Un indice d'attraction basé sur le temps passé dans chaque section a ensuite été calculé en suivant Hervé (2017). L'indice de chaque cultivar a été corrigé en soustrayant la valeur médiane globale du traitement témoin 'air pur' ce qui a contraint les indices dans l'intervalle [-1;1] où les valeurs négatives indiquent une " répulsion " et les valeurs positives une attraction. Chaque indice d'attraction corrigé a ensuite été comparé à zéro à l'aide d'un test de Wald appliqué à un modèle linéaire à effets mixtes où la plante individuelle était considérée comme un facteur aléatoire. Les résultats de ces tests sont présentés dans cette figure. NS : $p > 0,05$; * : $p < 0,05$. N = 30 femelles répondantes (i.e. sorties de la chambre d'introduction) par cultivar. A noter : pour acclimater les femelles avant l'expérience, celles-ci ont été placées dans une chambre d'introduction 10 min avant de connecter la chambre au tube en verre et de commencer les observations.

A. Les COV ubiquistes sont importants dans la discrimination de l'hôte

Malgré que l'attractivité respective des différents hôtes n'aient pas directement été mise en évidence dans ces travaux de thèse, les contrastes d'attractivité observés dans la littérature semblent confirmer que les signaux volatils sont en partie impliqués dans les préférences de choix des femelles, car l'espèce dont le bouquet de COV est le plus attractif est aussi l'espèce sur laquelle les femelles pondent le plus (Kergunteuil *et al.*, 2015b ; **article 1**). Une condition préalable à la capacité de faire un choix est la capacité de percevoir des espèces de plantes hôtes ou des populations comme différentes sur la base de leurs odeurs, et dépend de deux paramètres : les capacités perceptives des insectes et la présence d'informations sur l'identité des espèces dans les signaux volatils émis par les plantes (cf introduction générale). Généralement il est admis que différentes espèces de plantes ont des profils olfactifs moyens distincts par l'identité et les proportions des COV qu'ils contiennent (Van Den Boom *et al.*, 2004 ;

Schoonhoven *et al.*, 2005). Dans notre étude (**article 4**) nous avons pu montrer que les différents hôtes se discriminaient qualitativement par des combinaisons de composés ubiquistes. Aucune quantification des différents COV n'a été réalisée, ce qui aurait peut-être permis de discriminer les différents cultivars dont l'information identitaire doit certainement relever des proportions des composés plus que de leur identité. Le couplage des différentes techniques GC-EAD/FID avec la GC-MS, réalisée à notre connaissance pour la première fois sur *Delia radicum* avec des bouquets d'odeurs naturels, a permis de montrer que les femelles détectent des combinaisons spécifiques de ces composés ubiquistes qui sont fonction de l'espèce végétale. Ces résultats laissent penser qu'une partie de l'identité des espèces pourrait résider dans la combinaison de ces composés ; plus probablement encore dans le ratio ou la concentration des différents composés. N'ayant pas réalisé de quantification absolue de ces COV, nous ne pouvons pas conclure sur l'implication des ratios mais, leurs concentrations pourraient déjà être un premier signal identitaire. En effet, nous avons pu observer que l'α-farnésène par exemple n'est pas détecté par les antennes des femelles dans les bouquets naturels de *B. rapa* où il n'est présent qu'en faible concentration alors qu'il l'est dans les bouquets de *S. alba* et *B. oleracea* où les concentrations sont plus importantes. En plus de la présence d'information sur l'identité des espèces, nous avons montré que les femelles ont des capacités sensorielles qui leur permettent de détecter ces composés (et de nombreux autres) (**article 3**) et donc de pouvoir les utiliser au moins en partie pour discriminer les différents cultivars. Dans un grand nombre d'études qui visaient à identifier les signaux spécifiques impliqués dans le processus de sélection chez des insectes spécialistes des brassicacées, une approche analogue a été suivie en supposant que les glucosinolates (GLS) et leur dérivés volatils les isothiocyanates (ITC) étaient les seuls métabolites spécialisés importants à étudier à cause de leur occurrence chez cette famille de plantes (Hopkins *et al.*, 2009, Mithen *et al.*, 2010). Cela fut également le cas chez *D. radicum*, espèce pour laquelle l'influence des ITC dans le comportement d'orientation et d'acceptation de l'hôte a largement été décrit (Finch, 1978 ; Hawkes et Coaker, 1979 ; Wallbank et Wheatley 1979 ; Finch et Skinner, 1982b ; Nottingham et Coaker 1985, 1987 ; Nottingham, 1988 ; Tuttle *et al.*, 1988), contrairement à celle de composés plus ubiquistes (mais voir Kergunteuil *et al.*, 2012, 2015b ; Lamy *et al.*, 2017, 2018). Le choix de réaliser des captures d'odeurs de plantes saines (**article 4)** a contribué à minimiser la présence des ITC dans les bouquets, qui sont reconnus comme des signaux volatils importants dans l'attraction de l'hôte chez les spécialistes des brassicacées (exemples dans Wittstock *et al.*, 2003). Cependant, nous avons montré que malgré l'absence de composés spécifiques détectés dans les bouquets, les combinaisons des composés volatils plus ubiquistes contiendraient également des indices qui pourraient être utilisés par les femelles pour discriminer les différents hôtes et donc faire des choix. Il serait intéressant de réaliser une étude similaire en utilisant par exemple des odeurs de plantes prélevées en milieu naturel ou blessées au laboratoire. En effet, en utilisant des plantes saines, nous supprimons de l'équation toutes les autres odeurs naturellement produites suite aux différents stress biotiques et abiotiques que subissent les plantes (Loreto et Schnitzler, 2010 ; Gull *et al.*, 2019) et qui sont des signaux importants dans les interactions plantes-

insectes phytophages (Ameye *et al.*, 2018). Cependant, nous pouvons supposer que les signaux identitaires des différents hôtes sont relativement stables dans le temps pour permettre une reconnaissance sur le long terme, et que leur production ne serait pas fonction du type de stress subi par les plantes. Ainsi, il semble pertinent d'étudier si les bouquets de plantes saines transmettent des informations sur l'identité de l'espèce végétale bien que cela soit encore rarement fait dans la littérature (Conchou *et al.*, 2017).

B. Les COV peuvent agir indépendamment à courte distance ou au contact suivant la nature de leur combinaison

A travers les observations comportementales réalisées dans cette étude (**article 4**), nous avons également pu montrer que suivant la nature de la combinaison de COV proposée aux femelles, ils pouvaient avoir des effets indépendants sur les comportements pré- et post-atterrissage. En effet, certaines combinaisons semblaient attractives pour les femelles (effet à courte distance) tandis que d'autres semblaient plutôt stimuler leur comportement d'exploration de la feuille artificielle (effet au contact). Pour confirmer ces résultats, il serait nécessaire d'effectuer des observations comportementales supplémentaires avec plusieurs concentrations ou différents ratios de composés (idéalement les profils des bouquets émis naturellement par les plantes). En effet les concentrations ainsi que les proportions relatives des différents composés dans un bouquet d'odeurs sont des éléments essentiels de la spécificité des signaux chez les insectes phytophages (Bruce et Pickett, 2011 ; Tasin *et al.*, 2006 ; Salvagnin *et al.*, 2018).

Par ailleurs, ce travail souligne la complexité des effets des signaux volatils dans le processus d'acceptation de l'hôte en illustrant qu'avec seulement 4 composés et 3 combinaisons de ces composés, il est déjà possible d'observer différents comportements initiés de manière indépendante en fonction de la combinaison que ce soit à distance ou au contact. Il indique également l'importance de découpler les comportements pré- et post-atterrissage lors des observations pour comprendre de manière plus détaillée comment les signaux volatils modulent le comportement de choix chez les insectes phytophages.

2. Le signal au contact

A. Les composés de surface foliaire de tous les cultivars ne stimulent pas l'oviposition

Comme nous l'avons vu dans l'introduction, les composés de surface foliaire sont largement impliqués dans l'acceptation de l'hôte et dans les préférences d'oviposition des femelles phytophages, bien que cet aspect soit moins étudié. En effet, beaucoup d'études réalisées sur l'implication des composés de surface foliaire dans la stimulation de l'oviposition chez des femelles phytophages aériennes sont

réalisées à partir d'extraits de surface d'une variété ou d'un cultivar d'une seule espèce (ex : van Loon *et al.*, 1992 ; Udayagiri et Mason, 1997 ; Morris *et al.*, 2000 ; Lombarkia et Derridj, 2002 ; Howlett et Clarke, 2003 ; Maher et Thiery, 2004 ; Marazzi *et al.*, 2004 ; Gouinguené *et al.*, 2005). Peu d'études comparent l'effet de différentes espèces et/ou différents cultivars (mais voir par exemple Derridj *et al.*, 1996 ; Griffiths *et al.*, 2001 ; Justus *et al.*, 2000) alors qu'une telle comparaison parait pertinente pour comprendre les signaux impliqués dans le choix et donc la préférence des femelles. Les résultats de l'**article 5** ont permis de montrer que le message apporté par les composés de surface semble être un signal suffisant pour permettre aux femelles une discrimination plus fine à la fois entre deux espèces (*B. rapa* > *B. oleracea)* et entre différents cultivars d'une même espèce (cv. Tabaluga > cv. Richi). Cependant, bien qu'ils permettent une discrimination intraspécifique, ces signaux ne semblent pas être seuls à l'origine des préférences car le contraste observé uniquement à partir des composés de surface est inversé par rapport à celui observé sur plantes entières (cv. Richi > cv. Tabaluga) (**article 1**). De plus, le message chimique perçu par les femelles à la surface des feuilles n'est pas à l'origine de l'acceptation de tous les hôtes par les femelles. En effet, nous avons pu observer que les composés de surface foliaire de *B. rapa* et *B. oleracea* étaient suffisants pour induire la stimulation de l'oviposition contrairement à ceux de *S. alba* qui à eux seuls, ne semblaient pas apporter d'indices suffisants aux femelles pour stimuler leur oviposition. Ceci suggère une implication seulement partielle de ces composés dans la discrimination de l'hôte. Cette étude soulève l'importance de la comparaison entre différents hôtes avant de conclure sur la potentielle implication des composés de surface dans la sélection de l'hôte par les femelles phytophages.

B. Le profil particulier de S. alba

Dans la littérature, de nombreux composés de contact stimulant l'oviposition de *D. radicum* ont été identifiés, dont les '*Cabbage Identification Factors*' (CIF) et les GLS qui semblent avoir une forte activité stimulatrice (Baur *et al.*, 1996 ; Hurter *et al.*, 1999 ; de Jong *et al.*, 2000). Des dosages des GLS présents dans les feuilles des deux cultivars de *S. alba* au stade 2 feuilles ont été réalisés dans le cadre d'un projet mené dans l'équipe (Varialt, données non publiées) ont montré que seulement 2 GLS sur les 18 communément retrouvés chez les brassicacées étaient présents : la sinalbine (très largement majoritaire) et la glucotropaéoline. A notre connaissance, l'effet de la sinalbine sur la stimulation de l'oviposition n'est pas encore décrit dans la littérature. La glucotropaéoline peut être détectée par les sensilles gustatives tarsales des femelles et son effet sur la ponte des femelles est peu décrit mais ne semble pas dissuasif (Roessingh *et al.*, 1992). Ces dosages et d'autres réalisés par Tsunoda *et al.* (2017) montrent que la très grande majorité des GLS présents dans les feuilles de *S. alba* sont de type aromatique alors qu'il a été montré que les plus stimulants pour l'oviposition sont les GLS indoliques

(Roessingh *et al.*, 1992 ; Griffith *et al.*, 2001) qui eux sont largement retrouvés chez *B. rapa* et *B. oleracea* (Tsunoda *et al.*, 2017).

Les travaux que nous avons réalisés (**article 5**) ne permettent pas d'expliquer complètement l'acceptation des différents hôtes (notamment *S. alba*) et les préférences d'oviposition des femelles. Cependant, ils nous ont permis de nuancer la littérature existante sur l'implication des composés de surface dans l'acceptation de l'hôte chez les phytophages qui ne semble pas pouvoir se généraliser si facilement à l'échelle de toute la gamme d'hôte même lorsqu'elle est composée de plantes d'une même famille botanique qui ont pourtant des profils chimiques caractéristiques plus proches entre elles qu'avec les autres familles. Ce travail souligne la variabilité dans la nature des signaux impliqués dans la reconnaissance et remet en cause l'idée d'un signal universel, caractéristique à la famille qui pourrait être utilisé par les femelles dans la reconnaissance de l'hôte.

Les résultats des **articles 4** et **5** permettent de mieux comprendre les signaux et les mécanismes qui interviennent dans les préférences d'oviposition des femelles observées dans l'**article 1**. En effet, chez *B. rapa*, l'attraction des femelles à distance par les COV et la forte stimulation de l'oviposition par les composés de surface lors du contact avec la plante engendrerait *de facto* une acceptation forte de cet hôte et donc une large préférence pour celui-ci. Pour *S. alba*, les composés de surface ne semblent pas suffisants seuls pour induire l'oviposition mais les COV semblent pouvoir stimuler fortement l'exploration au contact et l'acceptation de cette espèce pourrait résulter d'une synergie entre les COV et les composés de surface. Cette hypothèse de la synergie entre ces deux signaux comme médiateur de l'acceptation de l'hôte par *D. radicum* a déjà été évoquée dans la littérature (de Jong et Städler, 1999). Pour la tester, nous avons tenté de mettre en place un dispositif dans lequel une feuille artificielle recouverte d'extrait de surface de moutarde était placée en présence d'une femelle dans une petite cage elle-même placée dans une plus grande cage dans laquelle se trouvaient des plantes entières de *S. alba* (Fig. 3). Ce dispositif permettait aux femelles d'avoir accès aux composés de surface à tester sur la feuille artificielle en étant soumise aux COV émis par une plante entière sans pouvoir rentrer en contact direct avec celle-ci. Plusieurs modalités ont été comparées : extraits de surface + COV, extrait de surface seuls, COV seuls ou rien (solvant d'extraction sur la feuille artificielle et absence de plante). Cependant, nous n'avons observé aucune ponte sur aucune des modalités. L'intérêt du dispositif n'a pas pu être validé car, même en proposant aux femelles des extraits de surface de *B. rapa* (seuls ou en combinaison avec des plantes entières), pourtant fortement stimulants (**article 3**), aucune ponte n'a pu être observée. De nombreux tests ont été réalisés afin de comprendre l'origine de cette non stimulation (en faisant varier l'âge des femelles, la paraffine, l'épaisseur de la couche qui en recouvre les feuilles, la nature du limbe de la feuille artificielle, le substrat de ponte) mais à l'heure actuelle, aucune explication n'a pu être apportée concernant l'absence de stimulation. Il serait intéressant de continuer à développer et à améliorer ce dispositif qui pourrait permettre de tester la complémentarité de deux signaux sur l'acceptation et la reconnaissance de l'hôte.

Figure 3 Photographie du dispositif expérimental qui visait à tester de manière combinée l'effet des composés de surface et l'effet des COV sur le comportement des femelles *D. radicum*.

II. Les préférences des femelles ne sont pas systématiquement corrélées avec la performance des larves

1. Pourquoi une corrélation positive ?

Chez les insectes phytophages, si le choix de l'hôte par la femelle est adaptatif au regard du succès du développement de sa descendance, alors la théorie évolutive prédit une corrélation positive entre la préférence d'oviposition des femelles et la performance des descendants (Gripenberg *et al.*, 2010). Le succès du développement des larves dépend à la fois de l'acceptation de la ressource et de sa qualité nutritive. Dans notre étude, une corrélation positive entre les préférences de ponte des femelles et une des variables de performance larvaire (définie par la taille du tibia et le temps de survie après émergence) a été observée à l'échelle intraspécifique chez *B. rapa.* Cependant, aucune différence dans l'acceptation par les larves, et qui pourrait être à l'origine du contraste de performance larvaire, n'a été mise en évidence (**article 2**). Ce contraste pourrait donc résulter d'une différence dans la qualité nutritive des cultivars de *B. rapa*. La teneur en protéines étant équivalente entre tous les cultivars testés sauf *B. oleracea* cv. Parthenon (Fig. 4a), elle ne semble pas à l'origine de la différence de qualité nutritive qui pourrait expliquer le contraste de performance. Hopkins *et al.* (1999) a pu montrer que chez *D. radicum*, la teneur en sucres des racines de *B. napus* et *B. oleracea* n'était pas positivement corrélée à la probabilité d'achever le développement larvaire. Nos résultats vont dans le sens de cette observation en

montrant les teneurs les plus faibles en sucres digestibles pour les deux cultivars de *B. rapa* (Fig. 4b), qui reste pourtant l'espèce qui confère la meilleure performance aux larves (**article 2**).

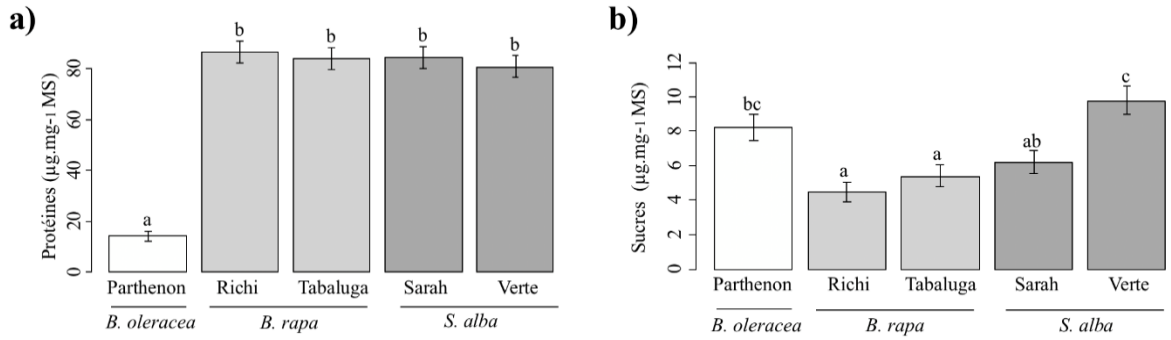

Figure 4 : Concentration moyenne (±ES ; µg.mg^{-1} MS) des **a)** protéines solubles totales et **b)** sucres digestibles totaux dans les racines des différents cultivars testés. Des lettres différentes indiquent des différences significatives entre les cultivars. N = 9 par cultivar (chaque échantillon est constitué des racines d'une à deux plantes). Dosage des protéines et des sucres d'après les protocoles de Deans *et al.* (2018) et Masuko *et al.* (2005) adapté d'après Laura Bellec.

2. Pourquoi les larves se développent mal sur les hôtes choisis par les femelles ?

Chez les insectes phytophages, la corrélation positive préférence/performance est plus généralement observée qu'une absence de corrélation ou une corrélation négative (Gripenberg *et al.*, 2010). Plusieurs hypothèses dans la littérature sont proposées pour expliquer l'observation d'une corrélation négative.

A. Le conflit mère/descendant

Tout d'abord, le comportement de choix des femelles n'est pas uniquement basé sur l'augmentation de la performance de leurs descendants. En effet, certaines femelles maximisent leur propre fitness en sélectionnant des hôtes de haute qualité pour se nourrir, et par conséquent optimisent leur nombre d'œufs produits plutôt que la performance de leurs descendants (Scheirs *et al.*, 2000). Chez *D. radicum*, les femelles pondent la plupart du temps sur des plantes jeunes (Santolamazza-Carbone *et al.*, 2017) qui ne leur offrent par conséquent pas de fleurs comme site d'alimentation. Le site d'alimentation étant différent du site d'oviposition, aucun conflit apparent pour les ressources n'existe entre les femelles et les larves. Il semble donc peu probable que la sélection de l'hôte par les femelles puisse être expliquée l'optimisation de leur propre performance.

B. Le rejet de l'hôte par la larve

Comme nous l'avons vu dans l'introduction, les larves sont capables d'accepter ou de rejeter un hôte sur lequel les femelles les ont préalablement déposées en se basant, comme les adultes, en grande partie sur les signaux chimiques qu'elles détectent. Cela semble être le cas ici chez *S. alba*, car les différentes expérimentations réalisées sur les larves ont montré que les racines du cultivar préféré par les femelles ne semblaient pas consommées par les larves contrairement aux racines d'un cultivar moins préféré pour l'oviposition. Le mauvais développement larvaire observé sur l'hôte préféré des femelles pourrait en partie être causé par un rejet de la plante par la larve. Ce rejet n'a pas pu être expliqué par un effet phagodissuasif strict des composés racinaires mais des effets contrastés de stimulation de l'alimentation ont pu être observés entre les deux cultivars (**article 2**). En plus des résultats présentés dans l'**article 2**, les dosages de protéines et de sucres ont permis de montrer que les racines du cultivar de *S. alba* non accepté par les larves contiennent une quantité de sucres plus faible que celles du cultivar accepté (Fig. 4b, respectivement 'Sarah' et 'Verte'), ce qui pourrait contribuer à la réduction de la phagostimulation mais n'explique pas totalement l'absence de consommation des racines. Les analyses métabolomiques des sous-fractions polaires contenant les composés biologiquement actifs sont en cours afin d'identifier des composés candidats impliqués dans la dissuasion ou l'absence de stimulation de l'alimentation, qui pourrait être à l'origine du rejet de la plante. En outre, une expérience permettant d'estimer la capacité de croissance compensatoire des racines du cultivar rejeté de *S. alba*, en réponse à la consommation des racines par les larves pourrait être pertinente afin d'écarter l'hypothèse d'une compensation de la part de la plante face aux attaques des larves, comme il a pu être observé chez d'autres espèces (Machado *et al.*, 2013 ; Hervé et Erb, 2019).

Notre étude (**article 2**) montre que pour tirer des conclusions sur la mesure dans laquelle les choix comportementaux reflètent une adaptation évolutive, il est pertinent de prendre en compte différentes variables de performance larvaire et de regarder cette corrélation à plusieurs échelles d'observation (**article 1**). En effet, si nous ne nous étions intéressés qu'à *B. rapa*, nous aurions pu supposer que la préférence des femelles était adaptative car elle est positivement corrélée avec la performance des descendants mais pour une seule variable de performance sur les trois testées. Cependant, aucune corrélation n'a été observée à l'échelle interspécifique et, une corrélation négative a été révélée à l'échelle intraspécifique chez *S. alba* ce qui remet en cause la validation de l'hypothèse de préférence-performance à l'échelle générale chez *D. radicum*. De plus, cette étude souligne que la validation de cette hypothèse n'est pas fonction de l'espèce de phytophage mais qu'elle peut dépendre de l'échelle d'observation.

Conclusion

Plus que d'avoir identifié des composés chimiques à l'origine des préférences d'oviposition de *D. radicum*, ce travail de thèse a permis de mettre évidence la complexité des signaux et des mécanismes impliqués dans la préférence des insectes phytophages. Les résultats obtenus dans ce travail ont montré qu'il est difficile d'établir des règles générales quant à la nature et l'effet des signaux utilisés par les insectes dans le processus de reconnaissance et de discrimination de l'hôte. En effet, la multiplicité des signaux chimiques émis par les plantes et la complexité de leurs effets sur le comportement des insectes rend difficile l'établissement de grands principes quant aux déterminants des schémas de préférence d'hôte chez les phytophages. Nos différentes études suggèrent que le signal utilisé dans l'acceptation peut ne pas être une caractéristique universelle de la gamme d'hôte, mais être beaucoup plus spécifique à chacun de ses hôtes. De plus, elles montrent que les insectes spécialistes ne sont pas forcément adaptés à répondre aux signaux caractéristiques de toutes les plantes de la famille végétale qu'ils exploitent, c'est pourquoi tirer des conclusions sur les signaux impliqués dans le choix des phytophages à partir de l'étude d'un seul hôte semble moins pertinent que de comparer les signaux utilisés entre différents hôtes. Cette thèse plaide ainsi pour l'intégration plus systématique des différentes échelles d'observation dans les interactions plantes-insectes.

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Communications scientifiques

Articles scientifiques

Menacer K, Cortesero AM, Hervé MR (2021) Challenging the Preference–Performance Hypothesis in an above-belowground insect. Oecologia 197:179–187.

Menacer K, Le Moal C, Ronzon E, Alletrut M, Cabon L, Lovillo E, Cortesero AM, Hervé MR (*in prep.*) Root compounds mediate host acceptance in larvae of an above-belowground specialist phytophagous insect

Menacer K, Hervé MR, Cortesero AM, Aujames T, Anton S (*in prep.*) Sex and maturity-dependent antennal detection of host plant volatiles in the cabbage root fly, *Delia radicum*

Menacer K, Lapeyre B, Vedrenne M, Hervé MR, Cortesero AM (*in prep.*) Host plant selection in phytophagous insects: volatiles can act independently at distance and at contact

Menacer K, Paty C, Hervé MR, Cortesero AM (*in prep.*) Host recognition mechanisms can vary within an insect herbivore's host range: oviposition is not always triggered by leaf surface compounds

Communications orales

Le nom de la personne ayant réalisé la présentation est souligné

Menacer K, Hervé MR, Cortesero AM (2021) Decrypting the mechanisms of host recognition and acceptance by a phytophagous insect. *2ème edition des Journées scientifiques de l'ED EGAAL*, Rennes, France.

Menacer K, Cortesero AM, Hervé MR (2021) Challenging the Preference-Performance Hypothesis in an above-belowground insect. *17th Symposium on Insect-Plant interactions,* Leiden, Netherlands.

Menacer K, Hervé MR, Cortesero AM (2021) Décrypter les mécanismes de reconnaissance au contact d'une plante hôte par un insecte phytophage spécialiste. *7èmes journées scientifiques du GDR MediatEC*, Toulouse, France.

Menacer K, Bellec L, Tixeront M, Hervé MR, Cortesero AM (2022) Determining contact chemical compounds responsible for host plant recognition in a belowground specialized phytophagous insect. *26th edition of the International Congress of Entomology,* Helsinki, Finlande.

Menacer K, Hervé MR, Cortesero AM (2022) Identifying the chemical determinants of host recognition at contact by phytophagous insects. *3ème édition des Journées scientifiques de l'ED EGAAL*, Rennes, Frrance

Posters

Menacer K, Cortesero AM, Hervé M (2019) Ceci n'est pas une plante hôte - Décrypter les mécanismes de reconnaissance et d'acceptation d'une plante par un insecte phytophage. *6èmes journées scientifiques du GDR MediatEC*, Lille, France.

Encadrement

Tous les stagiaires ont été supervisés par Maxime Hervé, Anne Marie Cortesero et Kathleen Menacer.

Stages obligatoires

Clémence Le Moal, M2, 2021 - Understanding the chemical bases of white mustard (*Sinapis alba*) resistance to larvae of the cabbage root fly (*Delia radicum*): a matter of acceptance?

Enzo Ronzon, M2, 2022 - Bases chimiques de la sélection de la plante-hôte chez la larve de la mouche du chou *Delia radicum*.

Manon Vedrenne, M1, 2022 - Etude de l'importance des composés de surface de feuilles sur la ponte de *Delia radicum*.

Stages volontaires

Lilian Cabon, M2, 2021 (1 mois) - Etude de l'influence des composés de contact sur l'acceptation de la plante par les femelles et les larves de *Delia radicum*.

Olivier Faloya, L1, 2021 (3 semaines) - Observations comportementales des femelles de la mouche du chou *Delia radium* sur un dispositif de feuille artificielle.

Maëlys Alletrut, L3, 2022 (3 mois) - Effet des bouquets d'odeurs de différents hôtes sur l'attraction des femelles de la mouche du chou *Delia radicum* en dispositif d'olfactométrie.

Vacations d'enseignement

2020/2021 : Université de Rennes 1, France

36h - UE Base de l'ECOlogie

4h - UE Evolution des ESPèces

2021/2022 : Université de Rennes 1, France

18h - UE Base de l'ECOlogie

12h - UE Evolution des ESPèces

UNIVERSITÉ DE RENNES 1

Titre : Décrypter les mécanismes de reconnaissance et d'acceptation d'une plante hôte par un insecte phytophage spécialiste

Mots clés : reconnaissance de l'hôte, composés chimiques volatils et de contact, interactions plantes-insectes, *Delia radicum*, Brassicaceae

Résumé : Chez les insectes phytophages, la nature des signaux à l'origine de la reconnaissance d'un hôte est largement décrite dans la littérature, contrairement à leur implication dans les préférences inter- et intraspécifiques. Nous avons étudié les signaux impliqués dans la reconnaissance et l'acceptation de trois espèces (*Brassica rapa*, *B. oleracea* et *Sinapis alba*) et populations de Brassicaceae hôtes par les femelles et les larves de la mouche du chou (*Delia radicum*). Les composés volatils ubiquistes émis par la partie aérienne de la plante semblent être impliqués dans la discrimination interspécifique. Nous avons également montré que selon leur combinaison, ils agissent indépendamment sur des comportements pré- et post-atterrissage des femelles. De plus, les composés de surface foliaire ne semblent pas être un signal de reconnaissance commun à tous les hôtes de la gamme, et sont impliqués dans les préférences intraspécifiques seulement chez *B. rapa*. Enfin, nous avons montré que les choix effectués par les femelles ne sont pas toujours corrélés avec la performance de leurs larves, qui peuvent rejeter, sur la base des composés racinaires, l'hôte préféré par les femelles mais seulement à l'échelle intraspécifique chez *S. alba*. L'ensemble de ces résultats mettent en lumière la grande complexité de la nature et des effets des signaux qui sont impliqués dans les préférences de l'hôte, en soulignant la nécessité d'intégrer une certaine diversité végétale dans les études sur les interactions entre les plantes et les insectes phytophages.

Title : Deciphering host plant recognition and acceptance mechanisms in a specialist phytophagous insect

Keywords : host recognition, volatile and contact chemicals, plant-insect interactions, *Delia radicum*, Brassicaceae

Abstract : In phytophagous insects, the nature of the cues responsible for host discrimination is widely described in the literature, contrary to their involvement in inter- and intraspecific preferences. We studied cues involved in the recognition and acceptation processes of three Brassicaceae species (*Brassica rapa*, *B. oleracea* and *Sinapis alba*) and populations by females and larvae of the cabbage root fly (*Delia radicum*). Ubiquitous volatile compounds emitted by the aerial part of the plant appear involved in the interspecific discrimination. We also showed that, depending on their combination, they act independently on pre- and post-landing behaviors . Furthermore, leaf surface compounds do not appear as recognition cues common to all host plants, and are involved in intraspecific preferences but only in *B. rapa*. Finally, we showed that females' choices are not always correlated with the performance of their larvae. Indeed, these appear to reject, based on root compounds, the host preferred by females but only at the intraspecific scale within *S. alba*. Taken together, these results highlight the high complexity of the nature and effects of cues involved in host preferences. This emphasizes the need to integrate more plant diversity in studies on interactions between plants and phytophagous insects.