COMMUNAUTÉ FRANÇAISE DE BELGIQUE

UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

ETUDE DES SÉMIOCHIMIQUES VOLATILS IMPLIQUÉS DANS LE MUTUALISME FOURMIS-PUCERONS

Christophe FISCHER

Dissertation originale présentée en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

Promoteur : Prof. Georges Lognay

Co-promoteur : Dr François Verheggen

2016

« Comment faire quatre triangles équilatéraux avec six allumettes ?… Il faut penser autrement »

B. Werber, Les Fourmis

COMMUNAUTÉ FRANÇAISE DE BELGIQUE

UNIVERSITÉ DE LIÈGE – GEMBLOUX AGRO-BIO TECH

ETUDE DES SÉMIOCHIMIQUES VOLATILS IMPLIQUÉS DANS LE MUTUALISME FOURMIS-PUCERONS

Christophe FISCHER

Dissertation originale présentée en vue de l’obtention du grade de docteur en sciences agronomiques et ingénierie biologique

Promoteur : Prof. Georges Lognay

Co-promoteur : Dr François Verheggen

2016

La réalisation de cette thèse a été rendue possible par une bourse du Fonds pour la formation à la Recherche dans l’Industrie et dans l’Agriculture (FRIA)

Fischer Christophe (2015) – Étude des sémiochimiques volatils impliqués dans le mutualisme fourmis-pucerons (Thèse de doctorat). Université de Liège, Gembloux Agro-Bio Tech, Gembloux, Belgique.

Résumé - Depuis des décennies, les relations mutualistes que peuvent présenter fourmis et pucerons passionnent les entomologistes, comme en atteste l'abondante littérature sur le sujet. Le principe de cette interaction est simple : aussi longtemps que leur colonie subsiste, les pucerons fournissent aux fourmis une source de sucres stable et abondante, le miellat, en échange de quoi ces dernières fournissent à leur partenaire entretien et protection contre divers ennemis naturels. Néanmoins, certains aspects de cette relation restent méconnus. C'est notamment le cas des facteurs influençant la recherche et de la découverte d'un partenaire potentiel, étape préalable à toute possibilité d'interaction mutualiste, et du rôle que tiennent les messagers chimiques volatils, généralement appelés sémiochimiques volatils, dans cette relation.

L'objectif de cette thèse est donc d'améliorer la compréhension du rôle tenu par les sémiochimiques volatils au sein de la relation mutualiste, et plus particulièrement dans l'étape de recherche de partenaire, tant du point de vue de la fourmi que de celui du puceron. La fourmi noire des jardins, *Lasius niger* L., et le puceron noir de la fève, *Aphis fabae* Scop., constituent le modèle d'étude.

L'impact des composés volatils sur le comportement de recherche de la fourmi a tout d'abord été investigué. Ces composés sont d'origines multiples, soit directement émis par le puceron, soit induits par sa présence. Premièrement, la capacité des fourmis à percevoir le E-β-farnésène, composé principal de la phéromone d'alarme de nombreux pucerons, et à s'en aider pour localiser une colonie de pucerons a été mise en évidence. Ce composé s'est en effet révélé attractif pour les fourmis, même à de faibles doses correspondant aux émissions d'une colonie de pucerons non stressée. Ensuite, le rôle tenu par les composés volatils du miellat dans l'attraction des fourmis a été démontré. Ces composés permettent également aux fourmis de discriminer à distance différentes espèces de pucerons et d'orienter leurs recherches en conséquence. Ces sémiochimiques sont produits par la microflore du miellat, et une souche bactérienne particulièrement attractive, *Staphylococcus xylosus*, a été isolée du miellat d'*A. fabae*.

Enfin, le rôle actif du puceron, et plus spécifiquement des formes ailées, les plus mobiles, dans la recherche d'un partenaire mutualiste a également été étudié. Ces derniers ne semblent pas s'orienter préférentiellement vers une plante hôte fréquentée par les fourmis ou voisine d'une fourmilière ; mais restent davantage sur la plante atteinte en présence de leur partenaire mutualiste.

Cette thèse met donc en évidence l'importance des sémiochimiques volatils et de la microflore du miellat dans les relations mutualistes fourmis-pucerons, complétant ainsi par un aspect nouveau un modèle biologique déjà bien documenté.

Fischer Christophe (2015) – Volatile semiochemicals involved in ant-aphid mutualism. (PhD thesis). University of Liège, Gembloux Agro-Bio Tech, Gembloux, Belgium.

Abstract - For decades, the mutualistic relationship that ant and aphids may present has fascinated entomologists, as evidenced by the abundant literature on the subject. The principles of this interaction are simple: as long as their colony remains, aphids provide ants with a stable and abundant source of sugars, honeydew. In exchange, ants tend aphid colonies and provide them cleaning and protection against various natural enemies. Nevertheless, some aspects of this relationship remain misunderstood. This is for example the case of the factors influencing the search and the discovery of a potential partner, first step to any potential mutualistic interaction. The role held by volatile chemical cues, called semiochemicals, in this relationship is also misunderstood.

The objective of this thesis is to improve understanding of the role played by volatile semiochemicals in this mutualistic relationship, especially during the partner search stage, both from the perspective of the ant and of the aphid. The black garden ant, *Lasius niger* L., and the black bean aphid, *Aphis fabae* Scop., constitute our biological model.

The impact of volatile compounds on the search behavior of the ant has first been investigated. These compounds are from multiple origins, either directly emitted by the aphid or induced by its presence. First, the ability of ants to perceive E-β-farnesene, the main component of many aphid alarm pheromone, and to use it to locate aphid colonies has been highlighted. This compound has indeed shown attractiveness to ants, even at low doses corresponding to the emissions of an unstressed aphid colony. Then, the roles played by honeydew volatile compounds in ant attraction have been demonstrated. These compounds also allow ants to remotely discriminate different aphid species and to direct their searches accordingly. These semiochemicals are produced by honeydew microflora, and a particularly attractive bacterial strain, *Staphylococcus xylosus*, has been isolated from *A. fabae* honeydew.

Finally, the active role of winged aphids, which are aphid's dispersal form, in the search for a mutualistic partner was also studied. Aphids do not appear to be preferentially attracted toward a host plant frequented by ants or close an ant nest. Nevertheless, once a potential host plant reached, they remain longer on it in case of ant presence.

This thesis highlights the importance of volatile semiochemicals and honeydew microflora in ant-aphid mutualistic relationships, thus completing by a new aspect an already well documented biological model.

REMERCIEMENTS

Au terme de cette thèse, je souhaite remercier tous ceux qui, de près ou de loin, ont contribué à sa réalisation.

Je souhaite avant tout adresser mes plus vifs remerciements à mon promoteur, Georges Lognay, sans qui cette thèse n’aurait pas vu le jour, pour ses exceptionnelles qualités tant humaines que scientifiques, pour son soutien tout au long de ce travail, ses conseils avisés, les discussions enrichissantes que nous avons partagées ainsi que la liberté de réflexion et de recherche qu’il m’a accordée.

Je souhaite également remercier François Verheggen, co-promoteur de ce travail, pour nos nombreuses discussions, ses conseils et explications en matière de publication, son aide précieuse lors de la rédaction du projet FRIA dans des délais très serrés, ainsi que pour m’avoir donné l’opportunité de participer à une formation extrêmement intéressante en écologie chimique à la Pennstate University (USA).

Ma reconnaissance va également à Claire Detrain, dont l’expertise en matière de mutualisme fourmis-pucerons fut particulièrement utile au cours de ce travail.

Je souhaiterais aussi remercier mon comité de thèse, qui a encadré ce travail depuis le début et a contribué à lui donner sa forme actuelle.

Je voudrais également adresser toute ma gratitude au Laboratoire de Chimie Analytique au complet, ainsi que ses occupants « à temps partiel », pour le soutien que j’y ai trouvé, les bons moments partagés, et l’excellente ambiance qui y règne ; à l’unité d’Entomologie Fonctionnelle et Evolutive, où ont pris place les élevages et tests éthologiques développés au cours de ce travail ; au BEAGx et aux unités de Chimie Générale et Organique et de Bioindustries, pour leur soutien matériel et technique ; ainsi qu’à tous ceux et celles avec qui j’ai pu collaborer dans le cadre de cette thèse ou de projets parallèles, et tout spécialement Bérénice, Danny, Delphine, Florence, Maryse, Pascal, Pierre, Vincent, ainsi que tous les étudiants stagiaires que j’ai pu encadrer.

Enfin, je souhaite remercier ma famille et mes amis, pour leur soutien et la motivation qu’ils m’ont apporté durant toute ma thèse, ainsi que tous ceux que je n’ai cités, mais qui se reconnaîtront.

CHAPITRE I

INTRODUCTION

Depuis des décennies, les relations mutualistes que peuvent présenter fourmis et pucerons passionnent les entomologistes, comme en atteste l'abondante littérature sur le sujet. Le principe de cette interaction est simple : aussi longtemps que leur colonie subsiste, les pucerons fournissent aux fourmis une source de sucres stable et abondante, le miellat, en échange de quoi ces dernières fournissent à leur partenaire entretien et protection contre divers ennemis naturels (coccinelles, syrphes, parasitoïdes, champignons pathogènes, …). Néanmoins, certains aspects de cette relation restent méconnus. C'est notamment le cas des facteurs influençant la recherche et de la découverte d'un partenaire potentiel, étape préalable à toute possibilité d'interaction mutualiste, et du rôle que tiennent les messagers chimiques volatils, généralement appelés sémiochimiques volatils, dans cette relation.

Après une introduction du contexte général dans lequel s'insère ce travail et un bref chapitre méthodologique, le rôle des sémiochimiques volatils utilisés par les fourmis pour s'aider dans la recherche d'un partenaire mutualiste sera étudié au travers de différentes questions.

Un premier point portera sur les composés volatils produits par le puceron lui-même, et plus spécifiquement aux phéromones couramment émises par les colonies de cet insecte, leur perception par un partenaire mutualiste potentiel, ainsi que leur effet sur le comportement de ce partenaire.

Ensuite, l'effet des volatils liés à la présence de pucerons, mais non directement émis par ces derniers, sur le comportement des fourmis sera investigué. En effet, la présence d'une colonie de pucerons sur une plante hôte induit également, outre les pucerons eux-mêmes, deux autres sources potentielles de composés volatils susceptibles de signaler leur présence: la plante hôte elle-même, potentiellement capable de modifier ses émissions de composés volatils en réponse à une attaque, ainsi que le miellat, qui s'accumule aux alentours de la colonie. Une attention particulière sera portée au miellat, élément clef du mutualisme par divers aspects, à l'origine des sémiochimiques volatils qu'il émet, ainsi qu'à leur impact sur l'attraction et la reconnaissance à distance d'un partenaire mutualiste.

Enfin, l'étendue du rôle actif du puceron dans la recherche d'un partenaire mutualiste sera étudiée.

Dans une dernière partie, les réponses apportées au cours de ce travail ainsi que les nouvelles pistes de réflexion ouvertes seront synthétisées et discutées.

CHAPITRE II

CONTEXTE

Avant-propos

De nombreuses revues et documents de référence couvrent déjà de manière exhaustive les divers aspects connus de la relation mutualiste fourmis-puceron ainsi que du rôle qu'y tient le miellat (El-Ziady and Kennedy 1956; Way 1963; Buckley 1987; Stadler and Dixon 1998; Stadler and Dixon 2005; Guénard *et al.* 2007; Stadler and Dixon 2008; Leroy *et al.* 2009; Yao 2014; Vilcinskas 2016). S'appuyant sur cette base, ce chapitre constitue une remise en contexte de la problématique du mutualisme fourmis-pucerons et de l'implication des sémiochimiques volatils dans la communication entre ces espèces. Les différentes questions abordées au cours de ce travail seront précédées d'introductions plus ciblées et complètes.

Le mutualisme : généralités et implications

Le mutualisme est une relation de coopération entre espèces, bénéfique pour les deux partenaires, pouvant aller d'interactions assez diffuses à des relations très étroites entre organismes. Ce type d'interaction est ubiquiste tant d'un point de vue géographique qu'évolutif, étant observé dans l'ensemble des écosystèmes, tant marins que terrestres, et touchant tous les règnes d'organismes vivants (Boucher *et al.*, 1982; Buckley, 1987; Guénard *et al.*, 2007). A titres d'exemple bien connu peut être citée la zoogamie, c'est à dire la pollinisation par le biais d'espèces animales. La plante assure ainsi sa reproduction, et le pollinisateur y trouve fréquemment une source de nourriture sous forme de nectar. Ce phénomène touche environ les trois quarts des espèces d'angiospermes, ainsi que de multiples taxa animaux. En effet, insectes, mais aussi oiseaux, chauves-souris et autres mammifères peuvent être impliqués dans ces relations (Kearns *et al.* 1998; Bronstein *et al.* 2006; Kremen *et al.* 2007; Suchet 2010). Autre exemple, dans un autre milieu : la relation entre anémones de mers et poisson-clowns (*Amphiprioninae),* les deux partenaires se protégeant mutuellement. La microflore peut également jouer un rôle actif dans les relations mutualistes, comme c'est le cas des *Rhizobiaceae* pouvant jouer un rôle majeur dans la fixation de l'azote chez les légumineuses. La bactérie induit la formation de nodosités au niveau racinaire, formant un microhabitat favorable à son développement, et fixe en échange l'azote atmosphérique sous une forme directement assimilable par la plante. Loin d'être exhaustifs, ces quelques exemples illustrent la diversité des taxa et milieux concernés par les relations mutualistes.

Les avantages retirés par chaque partenaire peuvent revêtir plusieurs formes de biens ou de services : nutrition, protection, reproduction, mobilité…, mais constituent généralement un coût pour l'autre partenaire. Cette relation peut donc être perçue comme de l'exploitation mutuelle globalement positive. Selon la majorité des modèles évolutifs actuels, le maintien d'une relation mutualiste ou son glissement vers d'autres types d'interactions se base sur une approche économique entre les coûts liés au maintien de la relation et les bénéfices qui en ressortent pour chacun des protagonistes (Bronstein *et al.* 2004; Bronstein *et al.* 2006; Guénard *et al.* 2007; Oliver *et al.* 2009; Herre *et al.* 2016). Dès lors, en fonction des conditions, cette relation peut se renforcer, ce qui est par exemple l'effet d'une forte pression de prédation sur un mutualisme fourmis-homoptères, s'estomper, ou évoluer vers du commensalisme, bénéfique pour seulement un seul des deux partenaires, voire du parasitisme (Guénard *et al.* 2007; Oliver *et al.* 2009). Outre les bénéfices et coûts directs pour les partenaires, l'importance de la sanction potentiellement infligée au partenaire qui sortirait de la relation, par exemple, la prédation, ainsi que le coût de la défection pour le partenaire lui-même, pouvant aller jusqu'à la mort dans le cas de mutualismes obligatoires, entrent également en ligne de compte pour le maintien d'une relation mutualiste (Guénard *et al.* 2007).

En effet, les différents partenaires peuvent être plus ou moins dépendants de cette relation. Le mutualisme peut être facultatif, lorsqu'il ne conditionne pas directement la survie des partenaires, ou obligatoire dans le cas contraire. Dans ce cas, l'obligation peut ne concerner qu'un seul des deux partenaires, l'autre restant dans un cas de mutualisme facultatif (Bronstein *et al.* 2004; Guénard *et al.* 2007). Dans tous les cas, ces relations mutualistes influencent sensiblement l'organisation des biosystèmes et ont un impact sur la diversité de nombreux taxa (Boucher *et al.* 1982; Styrsky and Eubanks 2007).

Le mutualisme fourmis-pucerons

Basée sur la production de nourriture en échange de services, la trophobiose est une forme de mutualisme largement répandue entre homoptères producteurs de miellat et fourmis. Ce type de mutualisme, probablement apparu pour la première fois durant le Tertiaire, s'est à de multiples reprises manifesté puis éteint dans des taxa distincts, et a probablement commencé par une relation prédateur-proie ayant évolué suite, entre autres, à la présentation de gouttelettes de miellat à l'assaillant (Stadler and Dixon 2005; Guénard *et al.* 2007).

Constituant l'un des principaux modèles de ce type d'interaction, le mutualisme fourmis-pucerons est l'une des relations mutualistes les plus étudiées du règne animal. En échange de la défense des colonies et de leur entretien, les pucerons fournissent aux fourmis une source de nourriture durable, abondante et appréciée : le miellat (Way 1963; Dixon 1985). Cette relation découle du mode de vie spécifique de chacun de protagonistes et des besoins inhérents.

Les pucerons vivent en colonies denses, qui peuvent atteindre un nombre important d'individus. Parmi les principales raisons expliquant ce mode de vie colonial figurent leur rapide moyen de reproduction parthénogénétique ainsi que la mobilité réduite des individus aptères constituant l'essentiel des colonies. Cette mobilité limitée, couplée à leurs faibles défenses, les désigne comme des proies faciles pour bon nombre de prédateurs et parasitoïdes (syrphes, coccinelles, guêpes parasitoïdes…) (Dixon 1985; Bristow 1991; Stadler and Dixon 2005; Lohman *et al.* 2006; Leroy *et al.* 2009).

Les pucerons se nourrissent de sève phloémienne, très riche en sucres par rapport aux autres nutriments nécessaires à leur entretien et leur reproduction. Conséquence de cette alimentation déséquilibrée, ils excrètent de grandes quantités de carbohydrates sous forme d'un liquide sucré, le miellat. Tombant à proximité des colonies, ce miellat constitue un risque pour l'hygiène et la santé de la colonie s'il s'y accumule. Il constitue en effet une source potentielle de développement de pathogènes ainsi qu'un signal de la présence de la colonie perceptible par de nombreux ennemis naturels du puceron tels les syrphes, coccinelles et guêpes parasitoïdes (Guénard *et al.* 2007; Leroy *et al.* 2009; Vilcinskas 2016). Comme pour toute autre société d'insecte et bien que rudimentaires par rapport à certaines de ces sociétés, l'organisation sociale et la communication au sein d'une colonie de pucerons, passe par le biais de la communication chimique (Kunert *et al.* 2005; Kunert *et al.* 2010).

Les fourmis, second partenaire, sont quant à elles parmi les insectes aux comportements sociaux les plus évolués. La communication chimique impliquée dans cette organisation sociale est également des plus complexes. Organisées, les colonies comportent une caste d'individus explorateurs, dont la fonction première est d'assurer la survie de la colonie en explorant l'environnement proche et en y exploitant les ressources disponibles. Cette exploitation, orientée par les besoins de la colonie et par les ressources disponibles, peut donner lieu à divers types de marquages chimiques tels que le marquage d'aire, à caractère territorial ou non, et le pistage (Devigne and Detrain 2002; Mailleux *et al.* 2003a; Devigne and Detrain 2006; Mailleux *et al.* 2006; Mailleux *et al.* 2010; Mailleux *et al.* 2011). Parmi les sources principales de nutriments, les plantes (et leurs sécrétions), les insectes et les fruits occupent une place importante (Wheeler 1926; Sudd 1967). Pour de nombreuses espèces de fourmis, le miellat excrété par les pucerons est également collecté comme source de sucres. Certaines espèces de fourmis deviennent alors de véritables éleveuses, exploitant la ressource miellat, tout en entretenant les colonies de pucerons, en veillant à leur hygiène et en les défendant contre leurs ennemis tant pathogènes que prédateurs et parasitoïdes. A l'occasion, en fonction des besoins de la colonie, de l'état des colonies de pucerons entretenues et du comportement individuel de ces pucerons, les fourmis peuvent prélever des pucerons de cet élevage en tant que source de protéines (El-Ziady and Kennedy 1956; Way 1963; Hölldobler and Wilson 1990; Stadler and Dixon 1999; Fischer *et al.* 2001; Yao and Akimoto 2001; Katayama and Suzuki 2003; Renault *et al.* 2005; Verheggen *et al.* 2009a; Nielsen *et al.* 2010). Elles sont également capables de reconnaître un puceron entretenu par la colonie d'un autre puceron, notamment par le biais d'hydrocarbures cuticulaires transférés au puceron au cours de son entretien (Glinwood *et al.* 2003; Endo and Itino 2012). Les pucerons myrmécophiles exploiteraient également les hydrocarbures cuticulaires, en présentant un profil caractéristique identifiable par les fourmis, pouvant être semblable à celui de ces dernières (Lang and Menzel 2011; Endo and Itino 2012; Yao 2014).

Cependant, bien que constituant un exemple classique fréquemment décrit dans la littérature (Buckley 1987), certains aspects de cette relation mutualiste restent méconnus, la grande majorité des données disponibles concernant des situations où les partenaires potentiels ou avérés sont déjà entrés en contact. Ainsi, certains facteurs importants pour conserver les soins fournis par les fourmis présentes, tels la concentration et la nature des sucres contenus dans le miellat, les quantités de miellat produites ainsi que le nombre de pucerons par fourmis, sont déjà bien décrits dans la littérature (Wheeler 1926; Sakata 1999; Mailleux et al. 2000; Yao and Akimoto 2001; Fischer et al. 2005; Mailleux et al. 2005; Stadler and Dixon 2005; Detrain et al. 2010). Mais il existe toujours très peu d'informations portant sur la recherche et la découverte de nouveaux partenaires potentiels et sur les facteurs influençant cette recherche.

La place du miellat dans la relation mutualiste

Source indirecte de composés volatils, et donc potentiellement impliqué dans les interactions distantes entre partenaires en plus de servir de monnaie d'échange, le miellat constitue un des éléments clefs du mutualisme fourmis-pucerons à divers égards. Sa composition ainsi que ses différents rôles et impacts sur les relations mutualistes fourmis-pucerons ont fait l'objet d'un important travail scientifique, synthétisé de manière assez complète au travers de plusieurs revues successives (Way 1963; Buckley 1987; Stadler and Dixon 2005; Guénard *et al.* 2007; Leroy *et al.* 2009).

Mélange complexe de sucres, d'acides organiques, d'acides aminés et de quelques lipides ; le miellat est excrété en grandes quantités lorsque les pucerons se nourrissent de sève phloémienne (Mittler 1958; Hussain *et al.* 1974; Leroy *et al.* 2009). Sa composition varie en fonction de différents facteurs liés entre autres au puceron, tels que l'espèce, l'âge et l'état physiologique, et à son environnement, tels que l'état physiologique de la plante hôte et les endosymbiontes du puceron (Fischer and Shingleton 2001; Fischer *et al.* 2002; Fischer *et al.* 2005; Guénard *et al.* 2007; Woodring *et al.* 2007; Leroy *et al.* 2009; Leroy *et al.* 2011b; Vilcinskas 2016). En effet, la composition de la sève phloémienne se reflète partiellement dans les sucres et acides aminés du miellat ; bien que d'autres acides aminés, dont certains essentiels, soient synthétisés par des bactéries endosymbiotiques pour pallier les déséquilibres et les faibles teneurs en ces composés observés dans la sève phloémienne (Douglas 1998; Febvay *et al.* 1999; Leroy *et al.* 2011b). Les pucerons sont également capables de produire d'eux-mêmes des sucres supplémentaires, dont le mélézitose (Mittler 1958; Kiss 1981; Woodring *et al.* 2007; Yao 2014; Vantaux *et al.* 2015). Cependant, d'autres paramètres peuvent également influencer la composition du miellat. C'est le cas de la présence de fourmis et de l'occurrence du mutualisme (Kiss 1981; Fischer and Shingleton 2001; Yao and Akimoto 2001; Guénard *et al.* 2007; Woodring *et al.* 2007). Illustrant bien la grande diversité de composition de cette sécrétion ainsi que sa variabilité, Leroy *et al.* 2009 proposent une revue de la composition du miellat en acides aminés et en sucres en fonction de l'espèce de puceron et de la plante hôte (Tableau 1)(Leroy *et al.* 2009). Cette composition en sucres ainsi que sa variabilité affectent les partenaires mutualistes à différents niveaux.

Ainsi, lorsqu'un choix entre plusieurs colonies de pucerons établies à proximité de la fourmilière se présente, les fourmis tendent à concentrer leur attention sur les colonies produisant le miellat le plus profitable, par son abondance, sa composition et sa teneur en

Tableau 1| Composition en sucres et en acides aminés de miellats excrétés par diverses espèces de pucerons se nourrissant sur différentes plantes hôtes

			Sucres														***Acides aminés***																		
Plante hôte	***Puceron***	***Myrmécophilie***	Erlose	Fructose	Glucose	Maltose	Maltotriose	Mannitol	Mélézitose	Mélibiose	Raffinose	Saccharose	Sorbitol	Tréhalose	Turanose	Xylose	Alanine	Arginine	Asparagine	Aspartate	Cystéine	Glutamate	Glutamine	Glycine	Histidine	Isoleucine	Leucine	Lysine	Méthionine	Phénylalanine	Proline	Sérine	Thréonine	Tryptophane	Valine
Q.q.	*Tuberculatus quercicola*[1]	f		x	x				x			x		x			x			x	x	x	x	x			x			x	x	x	x	x	x
S.t.	*Aulacorthum solani*[2]	n	x	x	x	x		x				x	x	x																					
	Macrosiphum euphorbiae[2]	f	x	x	x	x		x				x		x																					
	Myzus persicae[2]	n	x	x	x	x		x				x		x																					
T.a.	*M. persicae*[2]	n	x	x	x	x		x				x	x	x																					
	Sitobion avenae[2]	n	x	x	x	x		x				x	x	x																					
	Rhopalosiphum padi[2]	f	x	x	x	x		x	x	x		x	x	x																					
T.v.	*Metopeurum fuscoviride*[3]	f	x	x	x	x			x			x		x		x																			
	M. fuscoviride[4]	f		x	x	x			x		x	x				x																			
	M. fuscoviride[5]	f	x	x	x	x			x			x		x			x	x	x	x		x	x	x	x	x	x	x		x	x	x	x	x	x
	Brachycaudus cardui[4]	f		x	x	x			x		x	x				x																			
	B. cardui[5]	f	x	x	x	x			x			x		x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Trama troglodytes[5]	o	x	x	x	x			x			x		x			x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x
	Aphis vandergooti[5]	o	x	x	x	x			x			x		x			x	x	x	x	x	x	x		x	x	x		x	x	x	x		x	x
	Macrosiphoniella tanacetaria[4]	n			x							x				x																			
	M. tanacetaria[5]	n	x	x	x	x			x			x		x			x	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x
	Coloradoa tanacetina[5]	n	x	x	x	x			x			x		x			x	x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x
	Uroleucon tanaceti[5]	n	x	x	x	x			x			x		x			x		x	x		x	x	x	x	x	x		x	x	x	x	x	x	x
	Aphis fabae[5]	f	x	x	x	x			x			x		x			x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x
	A.fabae[4]	f		x	x	x						x				x																			
	A.fabae[6]	f	x	x	x	x			x			x		x		x																			
V.f.	*A.fabae*[7]	f		x	x	x			x	x	x	x		x		x																			
	A.fabae[8]	f	x	x	x	x	x		x			x		x																					
	A.fabae[6]	f	x	x	x	x			x			x		x																					
C.a.	*A.fabae*[6]	f	x	x	x	x			x			x		x	x																				
E.e.	*A.fabae*[6]	f	x	x	x	x			x		x	x		x																					

Plantes hôtes : *Q.q. : Quercus quercicola ; S.t. : Solanum tuberosum ; T.a. : Triticum aestivum ; T.v. : Tanecetum vulgare ; V.f. : Vicia faba ; C.a. : Chenopodium album ; E.e. : Euonymus europaeus.* **Myrmécophilie** : o : obligatoire ; f : facultative ; n : non myrmécophile.
[1] (Yao and Akimoto 2001); [2] (Hogervorst et al. 2007); [3] (Fischer et al. 2002); [4] (Völkl et al. 1999); [5] (Woodring et al. 2004); [6] (Fischer et al. 2005); [7] (Detrain et al. 2010); [8](Vantaux et al. 2011). Adapté de Leroy et al. (2009).

sucres (Kiss 1981; Hölldobler and Wilson 1990; Mailleux *et al.* 2000; Mailleux *et al.* 2003b; Detrain *et al.* 2010). Les interactions entre ces facteurs sont également à considérer. Ainsi, la richesse en sucre d'une source de miellat, constituée par le volume pondéré par la concentration, est un élément déterminant l'intensité de l'entretien d'une colonie (Fischer *et al.* 2005).

Ces éléments influent sur différents paramètres physiologiques tels l'appétence, la quantité ingérée, la digestibilité des sucres et l'impact général sur la survie de la fourmi, qui peuvent être reliés aux préférences montrées individuellement par les fourmis exploratrices et aux volumes ingérés (Völkl *et al.* 1999; Detrain *et al.* 2010), mais également avoir un impact au niveau du comportement de la colonie de par l'induction ou non de recrutement et de dépôt de pistes (Detrain *et al.* 2010). Une hiérarchisation des sucres couramment rencontrés dans le miellat a ainsi été observée chez *Lasius niger* L. tant au niveau de l'ingestion que du recrutement ; mélézitose, saccharose et raffinose étant préférés aux glucose et fructose, eux-mêmes préférés aux maltose et trehalose, alors que seuls les trois premiers induisent le dépôt de pistes (Detrain *et al.* 2010). Le mélézitose semble être un des sucres clefs, assez spécifique au miellat et particulièrement apprécié par les fourmis (Kiss 1981; Leroy *et al.* 2009; Detrain *et al.* 2010; Yao 2014). Sa teneur dans le miellat tend d'ailleurs à augmenter en cas d'interaction mutualiste (Fischer and Shingleton 2001; Yao and Akimoto 2001). Ce phénomène pourrait être l'un des paramètres liés à une coévolution visant à produire un miellat apprécié par le partenaire (Detrain *et al.* 2010). Les sucres jouent également en rôle de régulation de la pression osmotique dans le tractus digestif des pucerons. En effet, la synthèse d'oligosaccharides régule l'osmolalité chez les pucerons myrmécophiles. Pour ces derniers, l'ingestion de sève phloémienne augmente au-delà des besoins des insectes en cas d'entretien par les fourmis afin d'assurer une production de miellat suffisante (Kiss 1981; Fischer and Shingleton 2001; Yao and Akimoto 2001; Guénard *et al.* 2007; Woodring *et al.* 2007). Woodring *et al.* (2007) ont pu mettre en évidence que la synthèse d'importantes quantités de mélézitose et de moindres quantités de tréhalose par les enzymes digestives du puceron myrmécophile *Metopeurum fuscoviride* (Stroyan), concomitante à la rapide dégradation du saccharose en glucose et fructose, réduit l'osmolalité de l'intestin de ce puceron jusqu'au quart de ce qu'elle serait en l'absence de ces synthétases. Ce mécanisme n'est pas observé chez d'autres pucerons non myrmécophiles, dont *Macrosiphoniella tanacetaria* (Kaltenbach) et *Acyrthosiphon pisum* (Harris), produisant moins de miellat, et ne présentant donc pas la nécessité de synthétiser ces oligosaccharides pour réguler leur pression osmotique et attirer les fourmis (Woodring *et al.* 2007).

Contrairement à ce qui a longtemps été pensé, le miellat n'est donc pas qu'un simple déchet pour un puceron mutualiste et tant sa production que sa composition sont activement modifiées par ce dernier, ce qui représente un coût (Vantaux *et al.* 2015). Le mutualisme est une stratégie défensive où le puceron mutualiste cherche à réduire le coût global d'une rencontre avec un ennemi potentiel tel que la fourmi, sans chercher à éviter ces rencontres (Oliver *et al.* 2009). Au sein d'une même espèce, la production de miellat ainsi que sa composition et concentration en sucres peut significativement varier en fonction des générations, mais également entre clones (Fischer *et al.* 2005; Vantaux *et al.* 2011). Vantaux *et al.* (2011) ont ainsi observé d'énormes différences entre clones du puceron myrmécophile *Aphis fabae* en termes de production de mélézitose. Plus de la moitié des clones testés se sont révélés déficients en ce composé tant en présence qu'en absence de leur partenaire mutualiste. Ce type de comportement ne semble pas directement puni par le partenaire mutualiste qui collecte cependant moins fréquemment le miellat de ces clones, diminuant de ce fait sa présence à proximité de ces individus, et donc leur protection, mais également sa demande de miellat et le coût de cette surproduction pour le puceron. Les clones tricheurs peuvent ainsi partiellement bénéficier des avantages apportés par les fourmis attirées par les lignées non déficientes présentes sur la même plante sans pour autant en payer le coût (Vantaux *et al.* 2011; Vantaux *et al.* 2012).

Du point de vue du puceron, la balance coûts-bénéfices liée au mutualisme et aux modifications dans la production de miellat reste assez discutée dans la littérature, les résultats observés étant parfois contradictoires. En effet, là où certains auteurs attribuent au mutualisme un effet globalement positif sur le développement des colonies, le taux de reproduction, les capacités de vol et la survie générale de l'insecte (El-Ziady and Kennedy 1956; Johnson 1959; Guénard *et al.* 2007; Verheggen *et al.* 2009a), d'autres observent un coût lié à l'entretien par les fourmis, notamment sous la forme d'une réduction de taille des colonies et des insectes eux-mêmes, ainsi qu'une diminution des capacités de dispersion et de reproduction (Stadler and Dixon 1998; Stadler and Dixon 2005; Oliver *et al.* 2012; Tegelaar *et al.* 2012; Vantaux *et al.* 2015). Le bilan global de cette relation dépend fortement du contexte dans lequel elle se place (Stadler *et al.* 2001).

D'une part, l'effort constitué par la production de miellat en qualités et quantités satisfaisant une fourmi mutualiste est récompensé par une prédation par la fourmi fortement réduite ou nulle, ainsi qu'une protection contre d'autres ennemis naturels (Guénard *et al.* 2007; Nielsen *et al.* 2010; Yoo and Holway 2011; Yao 2012). Ceci constitue un intérêt non négligeable, particulièrement pour les espèces se nourrissant plus profondément dans la plante, aux pièces buccales plus longues et plus lentes à rétracter limitant les possibilités de fuite, ce qui explique la haute fréquence de mutualisme observée pour ces espèces (Shingleton *et al.* 2005). Avantage supplémentaire, les fourmis éliminent les compétiteurs non myrmécophiles de leurs partenaires mutualistes (Verheggen *et al.* 2009a; Powell and Silverman 2010).

D'autre part, le coût directement occasionné par les modifications dans la production de miellat liées à l'occurrence et à intensité de l'entretien par les fourmis peut s'avérer assez lourd : atrophie de l'appareil de vol, réduction de la taille du corps, survie jusqu'à maturité et longévité réduites à partir d'un certain niveau d'entretien… (Stadler and Dixon 1998; Stadler and Dixon 2005; Yoo and Holway 2011; Yao 2012; Yao 2014).

Le solde net de la balance des coûts et bénéfices liée aux modifications de la production de miellat est donc lié à l'environnement dans lequel la relation prend place.

Du point de vue de la fourmi, l'impact du miellat est nettement moins contrasté. Il constitue en effet une source de nourriture abondante et appréciée, impactant positivement la croissance des colonies par l'augmentation de la production de couvain et d'ouvrières liée à l'apport de sucres (Stadler and Dixon 2005; Guénard *et al.* 2007; Wilder *et al.* 2011; Shik *et al.* 2014). Cette ressource critique fait l'objet d'une compétition entre espèces locales, mais également avec certaines espèces invasives. C'est notamment le cas de la fourmi d'Argentine, *Linepithema humile (*Mayr), dont les propagules se nourrissant initialement essentiellement de proies riches en protéines ont évolué en supercolonies se nourrissant de miellat, ce régime apportant plus de bénéfices en termes de survie de la propagule et de croissance que les proies, même à faible densité de pucerons (Shik and Silverman 2013; Wilder *et al.* 2013; Shik *et al.* 2014).

Enfin, du point de vue des espèces gravitant autour de cette interaction mutualiste, le miellat a également son influence. Source de nourriture pour de nombreux ennemis naturels du puceron, le miellat agit également à la fois comme source de sémiochimiques volatils et de contact, pouvant influencer significativement les comportements des ennemis naturels du puceron, dont la recherche, la localisation et l'attaque de leur hôte ou proie ainsi que l'oviposition (Leroy *et al.* 2009). Leroy *et al.* (2009) proposent un tableau synthétique reprenant les différents effets du miellat sur divers ennemis naturels du puceron.

La place centrale du miellat au sein des interactions mutualistes n'est donc plus à établir, de nombreux aspects de cette problématique étant largement documentés. Cependant, bien que déjà mise en évidence en ce qui concerne les ennemis naturels du puceron, l'influence des composés volatils émis par le miellat sur le comportement du partenaire mutualiste reste à étudier.

Implication des sémiochimiques volatils dans la communication intra et interspécifique chez les partenaires mutualistes

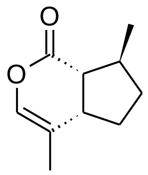
Bien que de complexités différentes, les organisations sociales et la communication au sein des colonies de fourmis et de pucerons passent entre autres par le biais de la communication chimique. Bien que la nature et les rôles primaires des composés impliqués soient des plus divers, ils constituent des indicateurs de la présence de l'insecte, voire même de son état physiologique ou de stress, potentiellement perceptibles à distance par d'autres, tant conspécifiques que prédateurs ou partenaires potentiels.

Plusieurs catégories de composés volatils ont déjà été identifiées chez le puceron. D'une part, les différents isomères de népétalactone (**I-II**) et de népétalactol (**III-VI**), phéromones sexuelles émises de manière ponctuelle au moment de la reproduction sexuée de l'insecte (Fig. 1) (Pickett *et al.* 1992; Goggin 2007; Pickett and Glinwood 2007; Verheggen 2008; Vilcinskas 2016)**.** Le mélange phéromonal observé chez la majorité des espèces de pucerons est généralement constitué d'un des énantiomères **I** et **II** de népétalactone, et/ou d'un des énantiomères **III** et **VI** de népétalactol, en proportions variables selon les espèces. Des stéréoisomères supplémentaires de népétalactol (**IV** et **V**) ont été identifiés chez le puceron du houblon, *Phorodon humuli* Schrank. La stéréochimie de ces composés ainsi que les ratios entre composés constituent des éléments majeurs expliquant la spécificité de l'action de ces mélanges phéromonaux (Vilcinskas 2016). Du fait de leur émission éphémère et intervenant en fin de saison, ils constituent des indicateurs de présence probablement peu intéressants dans le cadre d'une recherche de partenaire mutualiste.

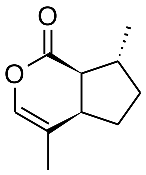
Figure 1 | Structure de sémiochiochimiques observés chez le puceron

Phéromones sexuelles

Népétalactones

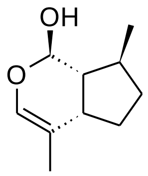


(I) (4aS,7S,7aR)-népétalactone

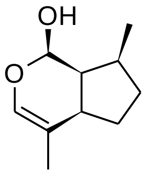


(II) (4aR,7R,7aS)-népétalactone

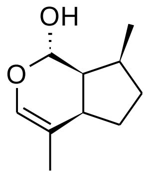
Népétalactols



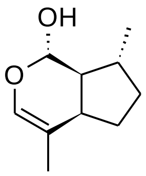
(III) (1R,4aS,7S,7aR)-népétalactol



(IV) (1R,4aR,7S,7aS)-népétalactol

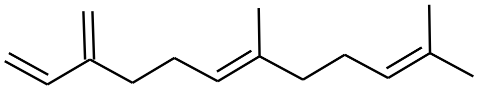


(V) (1S,4aR,7S,7aS)-népétalactol

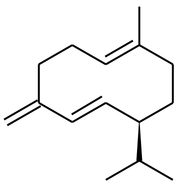


(VI) (1S,4aR,7R,7aS)-népétalactol

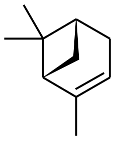
Autres phéromones



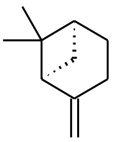
(VII) (E)-β-farnésène



(VIII) Germacrène D



(IX) α-pinène



(X) β-pinène

(Francis *et al.* 2005; Vilcinskas 2016)

Tableau 2| Composés organiques volatils détectés par SPME GC-MS dans 23 espèces de pucerons de comportements alimentaires différents et prélevés sur diverses plantes hôtes

Espèce de puceron :	**Récolté sur: (plantes hôtes)**	Régime alimentaire	Benzyl isothiocyanates	*α*-Bergamotene	*β*-Bourbonene	Camphene	p-Cimene	*α*-Copaene	(E)-*β*-farnesene	Germacrene D	*α*-Gurgunene	Isobornyl acetate	Limonene	Myrcene	*α*-Phellandrene	*β*-Phellandrene	*α*-Pinene	*β*-Pinene	*α*-Terpinene	Terpinolene	*α*-Thujene	ST204*	Unknown**
Acyrthosiphon pisum Harris	*Vicia faba* L.	SF	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aphis fabae Scop.	*Vicia faba* L.	P	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aphis idaei v.d.Goot	*Rubus sp.*	SE	-	-	-	-	6,4	-	1,3	-	-	-	-	15,0	0,9	16,0	6,0	**26,5**	18,3	-	8,4	-	-
Aphis sambuci L.	*Sambuscus nigra* L.	SG	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aulacortum solani Kalt.	*Sinapis alba* L.	P	-	-	-	-	-	-	**91,8**	-	-	-	-	-	-	-	-	3,0	-	-	-	5,2	-
Aphis urticata L.	*Urtica dioïca* L.	SE	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brachycaudus cardui L.	*Cynara scolymus* L.	P	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brachycaudus scharmtzi Börn	*Prunus persica* (L.) Batsch	SE	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brevicoryne brassicae	-	SF	**70,1**	-	-	-	-	-	5,0	-	9,4	-	-	-	-	-	-	2,0	-	-	-	1,7	5,6
Capitophorus elaeagui d.Guerc.	*Inula helenium* L.	SF	-	1,6	-	-	-	-	**73,1**	-	-	-	6,2	-	-	-	-	-	-	-	-	23,7	1,6
Chaitoporus populeti Panz.	*Populus alba* L.	SG	-	-	-	10,8	-	-	5,9	-	-	12,7	0,7	-	-	-	**53,3**	16,6	-	-	-	-	-
Dysaphis plantaginea Pass.	*Malus sylvestris* (L.) Mill	SG	-	-	-	-	-	-	15,9	-	-	-	-	-	-	-	-	-	-	-	-	**81,4**	-
Drepanosiphum platanoïdes Schr.	*Acer pseudoplatanus* L.	SG	-	-	-	1,2	-	-	-	-	-	1,1	32,8	-	-	8,2	12,8	**41,3**	-	0,7	-	-	-
Euceraphis punctipennis Zett.	*Betula pubescens* Ehrh.	SE	-	-	28,2	-	-	22,2	-	**42,0**	-	-	-	-	-	-	-	-	-	-	-	7,6	-
Hyalopterus pruni Geoffr.	*Prunus domestica* L.	SG	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hyperomyzus lactucae L.	*Sonchus arvensis* L.	SG	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macrosiphoniella tanacetaria Kalt.	*Tanacetum* sp	SG	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Megoura viciae L.	*Vicia faba* L.	SF	-	-	-	-	-	-	14,2	-	-	-	6,2	-	-	-	-	**74,0**	-	-	-	-	-
Metopolophium dirhodum Wlk.	*Triticum aestivum* L.	SF	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myzus cerasi Fabr.	*Prunus cerasus* L.	SE	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myzus lythri Schr.	*Prunus mahaleb* L.	SE	-	-	-	-	-	-	**83,3**	-	-	-	-	-	-	-	-	-	-	-	-	3,9	12,8
Myzus persicae Sultz.	*Vicia faba* L.	P	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sitobion avenae Fabr.	*Triticum aestivum* L.	SF	-	-	-	-	-	-	**100,0**	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Sesquiterpènes non identifiés ; ** molécules non identifiées ; - composé non détecté ; Résultats exprimés en pourcentage de l'aire totale.
Comportement alimentaire du puceron : P : espèce polyphage ; SF : espèce spécialiste d'une famille ; SG : espèce spécialiste d'un genre ; SE : espèce spécialiste d'une espèce

Adapté de Francis et al. (2005) et Holman (2009)

D'autre part, les pucerons peuvent produire une large gamme de terpènes, au sein de laquelle le E-β-farnésène (EBF) (**VII**) occupe une place prépondérante (Fig.1, Tableau 2). Cette molécule a en effet été identifiée dans 21 des 23 espèces étudiées par Francis *et al.* (2005), et constitue le composé majoritaire pour 16 d'entre elles. Le germacrène D (**VIII**), l'α-pinène (**IX**) et le β-pinène (**X**) sont les autres terpènes majoritaires observés par ces auteurs (Francis *et al.* 2005). Le E-β-farnésène constitue donc un sémiochimique clef, aux rôles multiples, et largement répandu chez les pucerons. Impliqué dans l'évaluation de l'environnement social d'un individu lorsqu'il est relâché continuellement en faible dose constante, l'EBF prend un rôle de phéromone d'alarme incitant les congénères à un comportement de fuite lorsqu'il est émis en pics de forte intensité par un individu soumis à l'attaque d'un ennemi naturel (Edwards *et al.* 1973; Turchin and Kareiva 1989; Francis *et al.* 2005; Verheggen *et al.* 2009b). Emis par l'insecte de manière régulière, l'EBF constitue potentiellement un bon indicateur de sa présence. Plusieurs études réalisées en conditions de laboratoire suggèrent d'ailleurs que divers ennemis naturels du puceron, dont des coccinelles, des syrphes et des chrysopes, sont capables de détecter ce composé et de s'en aider afin de localiser leur proie ou leur hôte (Micha and Wyss 1996; Zhu *et al.* 1999; Verheggen *et al.* 2007; Verheggen *et al.* 2009b; Durieux *et al.* 2010; Outreman *et al.* 2010; Verheggen *et al.* 2012). Dans le cadre des interactions avec les fourmis, Nault *et al.* (1976) ont démontré la capacité des fourmis à détecter de fortes doses d'EBF et à y réagir. Les fourmis adoptent alors un comportement agité et agressif, suggérant une réaction de défense d'un partenaire mutualiste soumis à une attaque (Nault *et al.* 1976). Cependant, la capacité des fourmis à détecter de faibles niveaux d'émission d'EBF, correspondant à son taux d'émission en l'absence d'attaque, et à s'en aider afin de localiser un partenaire mutualiste reste à démontrer.

Outre ces phéromones directement émises par le puceron, d'autres sémiochimiques peuvent également être induits par la présence de l'insecte. En effet, en réponse à l'attaque de pucerons, diverses plantes peuvent modifier leurs émissions de composés volatils. Ces changements peuvent être perçus par les plantes environnantes, pouvant alors préparer leurs défenses contre les pucerons, mais également par les ennemis naturels du puceron (Kost and Heil 2006; Paris *et al.* 2010; Staudt *et al.* 2010). Autre source de composés volatils induite par la présence de pucerons, le miellat produit par les colonies et s'accumulant aux abords de la plante hôte est également connu pour attirer les ennemis naturels du puceron. Les volatils qu'il émet proviendraient d'une part de l'altération chimique (oxydation…) des sucres et acides aminés contenus, influencée entre autres par des facteurs abiotiques tels que la lumière, l'humidité relative et la température, et d'autre part de l'action de la microflore sur le miellat (Leroy *et al.* 2009; Leroy *et al.* 2011a). Seuls ou couplés aux constituants non volatils du miellat, ces composés sont connus pour influencer les comportements de recherche d'hôte ou de proie et d'oviposition de divers parasitoïdes et prédateurs de pucerons, incluant à nouveau diverses espèces de coccinelles, syrphes et guêpes parasitoïdes (Aphidiinae) (Leroy *et al.* 2009). Néanmoins, bien que l'impact de ces volatils indicateurs indirects de la présence de pucerons sur leurs ennemis naturels soit déjà bien documenté, leur rôle dans les relations mutualistes reste encore largement à étudier.

Concernant les fourmis, le nombre, la diversité et les fonctions des composés connus impliqués dans leur communication intraspécifique sont bien supérieurs, et largement influencés par l'espèce. Ces sémiochimiques ; sécrétés par diverses glandes telles les glandes mandibulaires, à poison, sternale, pygidiale, métapleurale, de Dufour… (plus de 75 glandes exocrines sont connues chez les Formicidae (Billen 2009)) ; contribuent à des aspects variés des interactions au sein des colonies, dont les comportements d'alarme, la reconnaissance et l'appartenance à une colonie, le marquage du territoire et du nid, ainsi que le dépôt de pistes. Leur nature ainsi que leur degré de volatilité varient également fortement, ces composés incluant notamment hydrocarbures, cétones, aldéhydes, alcools, acides carboxyliques et esters de différentes longueurs de chaînes, linéaires ou cycliques, ramifiés ou non ; terpénoïdes divers ; pyrazines substituées et autres composés azotés cycliques ou non (Parry and Morgan 1979; Attygalle and Morgan 1985; Morgan 2008; Cardé and Millar 2009; Morgan 2009).

Bien qu'il soit communément admis que le partenaire le plus actif dans l'établissement d'une relation mutualiste soit la fourmi, il est possible qu'un puceron myrmécophile, et plus spécifiquement sa forme ailée, la plus mobile, soit capable d'utiliser ces sémiochimiques afin de s'établir préférentiellement sur une plante hôte fréquentée par un potentiel partenaire mutualiste ou à proximité d'une fourmilière. Ceci constituerait un avantage non négligeable pour un puceron myrmécophile obligatoire ou facultatif, qui représentent chacun environ un tiers des espèces européennes (Stadler *et al.* 2001). Divers auteurs rapportent d'ailleurs l'impact des fourmis sur la structure et la répartition des colonies de pucerons, notant une abondance accrue de pucerons dans les zones fréquentées par leurs partenaires mutualistes. La question de savoir si ce dernier phénomène est lié à un meilleur taux de survie des pucerons dans ces conditions ou à leur établissement préférentiel dans ces zones reste ouverte (Hopkins and Thacker 1999; Bishop and Bristow 2003; Stewart-Jones *et al.* 2008; Minarro *et al.* 2010; Novgorodova and Gavrilyuk 2012). Il est à noter que, les possibilités de vol directionnel des formes ailées étant souvent limitées et que leurs déplacements sur de longues distances étant fortement influencés par les courants aériens (Stadler *et al.* 2001; Guénard *et al.* 2007), la sélection d'un site pourrait être significativement influencée par la rétention d'un puceron sur un site propice plutôt que son vol délibéré vers ce site.

Tant pour les fourmis que les pucerons, les sémiochimiques volatils jouent donc un rôle de premier plan dans la perception de leur environnement social et leurs interactions intraspécifiques, ainsi que dans la manière dont ces insectes sont perçus et interagissent avec d'autres espèces. Au cours de ce travail, le rôle des composés volatils émis ou induits par la présence d'un des partenaires mutualistes sur le comportement de l'autre insecte sera investigué.

Malgré la diversité et le nombre de recherches déjà menées sur le mutualisme fourmis-pucerons, certains pans de cette relation restent peu connus. C'est notamment le cas des mécanismes impliquant des sémiochimiques volatils dans la recherche à distance d'un partenaire, auxquels une attention particulière sera portée au cours de ce travail. Cette approche originale d'un modèle biologique déjà largement documenté permettra d'améliorer la compréhension d'aspects importants du mutualisme que sont la recherche de partenaire et les éléments sur lesquels se base cette recherche.

Références

Attygalle AB, Morgan ED (1985) Ant trail pheromones. Adv In Insect Phys 18:1–30.

Billen J (2009) Diversity and Morphology of Exocrine Glands in Ants. In: XIX Simposio de Mirmecologia. Ouro Preto (Brasil), pp 1–6

Bishop DB, Bristow CM (2003) Effects of the presence of the Allegheny mound ant (Hymenoptera : Formicidae) in providing enemy-free space to myrmecophilous aphid and soft scale populations. Ann Entomol Soc Am 96:202–210.

Boucher DH, James S, Keeler KH (1982) The ecology of mutualism. Annu Rev Ecol Syst Vol 13 315–347.

Bristow CM (1991) Why are so few aphids ant-tended? In: Cutler DF, Huxley C (eds) Ant/Plant Interactions. Oxford University Press, pp 104–119

Bronstein JL, Alarcon R, Geber M (2006) The evolution of plant-insect mutualisms. New Phytol 172:412–428. doi: 10.1111/j.1469-8137.2006.01864.x

Bronstein JL, Dieckmann U, Ferrière R (2004) Coevolutionary Dynamics and the Conservation of Mutualisms. Evol Conserv Biol 305–326.

Brysch-Herzberg M (2004) Ecology of yeasts in plant-bumblebee mutualism in Central Europe. FEMS Microbiol Ecol 50:87–100. doi: 10.1016/j.femsec.2004.06.003

Buckley R (1987) Ant-Plant-Homopteran Interactions. In: Macfadyen A, Ford ED (eds) Advances in Ecological Research. Academic Press, pp 53–85

Cardé RT, Millar JG (2009) Chapter 195 - Pheromones. In: Cardé VHRTBT-E of I (Second E (ed) Encyclopedia of Insects. Academic Press, San Diego, pp 766–772

Detrain C, Verheggen FJ, Diez L, et al (2010) Aphid-ant mutualism: how honeydew sugars influence the behaviour of ant scouts. Physiol Entomol 35:168–174.

Devigne C, Detrain C (2002) Collective exploration and area marking in the ant Lasius niger. Insectes Soc 49:357–362.

Devigne C, Detrain C (2006) How does food distance influence foraging in the ant Lasius niger: The importance of home-range marking. Insectes Soc 53:46–55. doi: 10.1007/s00040-005-0834-9

Dixon AFG (1985) Aphid ecology. Blackie ; Distributed in the U.S.A. by Chapman and Hall, Glasgow

Douglas AE (1998) Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria Buchnera. Annu Rev Entomol 43:17–37. doi: 10.1146/annurev.ento.43.1.17

Durieux D, Verheggen FJ, Vandereycken A, et al (2010) Review: chemical ecology of ladybird beetles. Biotechnol Agron Soc Environ 14:351–367.

Edwards LJ, Siddall JB, Dunham LL, et al (1973) Trans-β-farnesene, alarm pheromone of the green peach aphid, Myzus persicae (Sulzer) [15]. Nature 241:126–127. doi: 10.1038/241126b0

El-Ziady S, Kennedy JS (1956) Beneficial effects of the common garden ant, Lasius niger L., on the black bean aphid, Aphis fabae Scopoli. Proc R Ent Soc Lond 31:5.

Endo S, Itino T (2012) Myrmecophilous aphids produce cuticular hydrocarbons that resemble those of their tending ants. Popul Ecol 1–8. doi: 10.1007/s10144-012-0355-0

Febvay G, Rahbe Y, Rynkiewicz M, et al (1999) Fate of dietary sucrose and neosynthesis of amino acids in the pea aphid, acyrthosiphon pisum, reared on different diets. J Exp Biol 202 (Pt 19:2639–2652.

Fischer MK, Hoffmann KH, Völkl W (2001) Competition for mutualists in an ant-homopteran interaction mediated by hierarchies of ant attendance. Oikos 92:531–541.

Fischer MK, Shingleton AW (2001) Host plant and ants influence the honeydew sugar composition of aphids. Funct Ecol 15:544–550.

Fischer MK, Volkl W, Hoffmann KH (2005) Honeydew production and honeydew sugar composition of polyphagous black bean aphid, Aphis fabae (Hemiptera: Aphididae) on various host plants and implications for ant-attendance. Eur J Entomol 102:155–160.

Fischer MK, Volkl W, Schopf R, Hoffmann KH (2002) Age-specific patterns in honeydew production and honeydew composition in the aphid Metopeurum fuscoviride: implications for ant-attendance. J Insect Physiol 48:319–326.

Francis F, Vandermoten S, Verheggen F, et al (2005) Is the (E)-β-farnesene only volatile terpenoid in aphids? J Appl Entomol 129:6–11.

Glinwood R, Willekens J, Pettersson J (2003) Discrimination of aphid mutualists by an ant based on chemical cues. Acta Agric Scand Sect B-Soil Plant Sci 53:177–182. doi: Doi 10.1080/09064710310015445

Goggin FL (2007) Plant-aphid interactions: molecular and ecological perspectives. Curr Opin Plant Biol 10:399–408. doi: 10.1016/j.pbi.2007.06.004

Guénard B, Lucas É, Francoeur A (2007) Mutualisme fourmis pucerons et guilde aphidiphage associée : le cas de la prédation furtive. Université du Québec à Montréal

Herre EA, Knowlton N, Mueller UG, Rehner SA (2016) The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends Ecol Evol 14:49–53. doi: 10.1016/S0169-5347(98)01529-8

Hölldobler B, Wilson EO (1990) The Ants. Springer-Verlag GmbH

Hopkins GW, Thacker JI (1999) Ants and habitat specificity in aphids. J Insect Conserv 3:25–31.

Hussain A, Forrest JM, Dixon AF (1974) Sugar, organic acid, phenolic acid and plant growth regulator content of extracts of honeydew of the aphid Myzus persicae and of its host plant, Raphanus sativus. Ann Appl Biol 78:65–73. doi: 10.1111/j.1744-7348.1974.tb01486.x

Johnson B (1959) Ants and form reversal in aphids. Nature 184:740–741. doi: 10.1038/184740a0

Katayama N, Suzuki N (2003) Bodyguard effects for aphids of Aphis craccivora Koch (Homoptera : Aphididae) as related to the activity of two ant species, Tetramorium caespitum Linnaeus (Hymenoptera : Formicidae) and Lasius niger L. (Hymenoptera : Formicidae). Appl Entomol Zool 38:427–433.

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: The conservation of plant-pollinator interactions. Annu Rev Ecol Syst 29:83–112. doi: 10.1146/annurev.ecolsys.29.1.83

Kiss A (1981) Melezitose, aphids and ants. Oikos 37:382.

Kost C, Heil M (2006) Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. J Ecol 94:619–628.

Kremen C, Williams NM, Aizen M a., et al (2007) Pollination and other ecosystem services produced by mobile organisms: A conceptual framework for the effects of land-use change. Ecol Lett 10:299–314. doi: 10.1111/j.1461-0248.2007.01018.x

Kunert G, Otto S, Röse USR, et al (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecol Lett 8:596–603.

Kunert G, Reinhold C, Gershenzon J (2010) Constitutive emission of the aphid alarm pheromone, (E)-beta-farnesene, from plants does not serve as a direct defense against aphids. BMC Ecol 10:(23 November 2010)–(23 November 2010).

Lang C, Menzel F (2011) Lasius niger ants discriminate aphids based on their cuticular hydrocarbons. Anim Behav 82:1245–1254. doi: http://dx.doi.org/10.1016/j.anbehav.2011.08.020

Leroy P, Capella Q, Haubruge E (2009) Aphid honeydew impact on the tritrophic relationships between host-plants, phytophagous insects and their natural enemies. Biotechnol Agron Soc Environ 13:325–334.

Leroy PD, Sabri A, Heuskin S, et al (2011a) Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. Nat Commun 2:7. doi: Artn 348 Doi 10.1038/Ncomms1347

Leroy PD, Wathelet B, Sabri A, et al (2011b) Aphid-host plant interactions: does aphid honeydew exactly reflect the host plant amino acid composition? Arthropod Plant Interact 5:193–199. doi: DOI 10.1007/s11829-011-9128-5

Lohman DJ, Liao Q, Pierce NE (2006) Convergence of chemical mimicry in a guild of aphid predators. Ecol Entomol 31:41–51. doi: 10.1111/j.0307-6946.2006.00758.x

Mailleux AC, Buffin A, Detrain C, Deneubourg JL (2011) Recruitment in starved nests: The role of direct and indirect interactions between scouts and nestmates in the ant Lasius niger. Insectes Soc 58:559–567. doi: 10.1007/s00040-011-0177-7

Mailleux AC, Deneubourg JL, Detrain C (2003a) Regulation of ants' foraging to resource productivity. Proc R Soc London Ser B-Biological Sci 270:1609–1616. doi: DOI 10.1098/rspb.2003.2398

Mailleux AC, Deneubourg JL, Detrain C (2000) How do ants assess food volume? Anim Behav 59:1061–1069.

Mailleux AC, Deneubourg JL, Detrain C (2003b) How does colony growth influence communication in ants? Insectes Soc 50:24–31. doi: 10.1007/s000400300004

Mailleux AC, Detrain C, Deneubourg JL (2006) Starvation drives a threshold triggering communication. J Exp Biol 209:4224–4229. doi: 10.1242/jeb.02461

Mailleux AC, Detrain C, Deneubourg JL (2005) Triggering and persistence of trail-laying in foragers of the ant Lasius niger. J Insect Physiol 51:297–304. doi: 10.1016/j.jinsphys.2004.12.001

Mailleux AC, Devigne C, Deneubourg JL, Detrain C (2010) Impact of starvation on lasius niger' exploration. Ethology 116:248–256. doi: 10.1111/j.1439-0310.2009.01736.x

Micha SG, Wyss U (1996) Aphid alarm pheromone (E)-β-farnesene: a host finding kairomone for the aphid primary parasitoid Aphidius uzbekistanicus (Hymenoptera: Aphidiinae). Chemoecology 7:132–139.

Minarro M, Fernandez-Mata G, Medina P (2010) Role of ants in structuring the aphid community on apple. Ecol Entomol 35:206–215. doi: DOI 10.1111/j.1365-2311.2010.01173.x

Mittler TE (1958) The feeding and nutrition of large willow aphid (Tuberolachnus salignus). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. J Exp Biol 35:74–84.

Morgan ED (2009) Trail pheromones of ants. Physiol Entomol 34:1–17. doi: 10.1111/j.1365-3032.2008.00658.x

Morgan ED (2008) Chemical sorcery for sociality: Exocrine secretions of ants (Hymenoptera: Formicidae). Myrmecological News 11:79–90.

Nault LR, Montgomery ME, Bowers WS (1976) Ant-aphid association: role of aphid alarm pheromone. Sci USA 192:1349–1351.

Nielsen C, Agrawal AA, Hajek AE (2010) Ants defend aphids against lethal disease. Biol Lett 6:205–208. doi: DOI 10.1098/rsbl.2009.0743

Novgorodova TA, Gavrilyuk A V (2012) The degree of protection different ants (Hymenoptera: Formicidae) provide aphids (Hemiptera: Aphididae) against aphidophages. Eur J Entomol 109:187–196.

Oliver TH, Leather SR, Cook JM (2009) Tolerance traits and the stability of mutualism. Oikos 118:346–352. doi: DOI 10.1111/j.1600-0706.2008.17045.x

Oliver TH, Leather SR, Cook JM (2012) Ant larval demand reduces aphid colony growth rates in an ant-aphid interaction. Insects 3:120–130. doi: 10.3390/insects3010120

Outreman Y, Kunert G, Simon J-C, Weisser W (2010) Ecological Costs of Alarm Signalling in Aphids. In: Kindlmann P, Dixon AFG, Michaud JP (eds) Aphid Biodiversity under Environmental Change SE - 10. Springer Netherlands, pp 171–181

Paris CI, Llusia J, Peñuelas J (2010) Changes in monoterpene emission rates of quercus ilex infested by aphids tended by native or invasive lasius ant species. J Chem Ecol 36:689–698. doi: 10.1007/s10886-010-9815-1

Parry K, Morgan ED (1979) Pheromones of ants: a review. Physiol Entomol 4:161–189. doi: 10.1111/j.1365-3032.1979.tb00193.x

Pickett JA, Glinwood RT (2007) Chemical ecology. In: Aphids as Crop Pests. Biological Chemistry Division, Rothamsted Research, Harpenden, Herts, AL5 2JQ, United Kingdom, pp 235–260

Pickett JA, Wadhams LJ, Woodcock CM, Hardie J (1992) The chemical ecology of aphids. Annu Rev Entomol 67–90.

Powell BE, Silverman J (2010) Population Growth of Aphis gossypii and Myzus persicae (Hemiptera: Aphididae) in the Presence of Linepithema humile and Tapinoma sessile (Hymenoptera: Formicidae). Environ Entomol 39:1492–1499. doi: Doi 10.1603/En09211

Renault CK, Buffa LM, Delfino MA (2005) An aphid-ant interaction: effects on different trophic levels. Ecol Res 20:71–74. doi: DOI 10.1007/s11284-004-0015-8

Sakata H (1999) Indirect interactions between two aphid species in relation to ant attendance. Ecol Res 14:329–340.

Shik JZ, Kay AD, Silverman J (2014) Aphid honeydew provides a nutritionally balanced resource for incipient Argentine ant mutualists. Anim Behav 95:33–39. doi: 10.1016/j.anbehav.2014.06.008

Shik JZ, Silverman J (2013) Towards a nutritional ecology of invasive establishment: aphid mutualists provide better fuel for incipient Argentine ant colonies than insect prey. Biol Invasions 15:829–836. doi: 10.1007/s10530-012-0330-x

Shingleton AW, Stern DL, Foster WA (2005) The origin of a mutualism: A morphological trait promoting the evolution of ant-aphid mutualisms. Evolution (N Y) 59:921–926.

Stadler B, Dixon AFG (1998) Costs of ant attendance for aphids. J Anim Ecol 67:454–459.

Stadler B, Dixon AFG (2005) Ecology and evolution of aphid-ant interactions. Annu Rev Ecol Evol Syst 36:345–372.

Stadler B, Dixon AFG (2008) Mutualism: Ants and their Insect Partners. Department of Animal Ecology, Bayreuth Institute for Terrestrial Ecosystem Research, University of Bayreuth, Germany

Stadler B, Dixon AFG (1999) Ant attendance in aphids: why different degrees of myrmecophily? Ecol Entomol 24:363–369.

Stadler B, Fiedler K, Kawecki TJ, Weisser WW (2001) Costs and benefits for phytophagous myrmecophiles: When ants are not always available. Oikos 92:467–478.

Staudt M, Jackson B, El-Aouni H, et al (2010) Volatile organic compound emissions induced by the aphid Myzus persicae differ among resistant and susceptible peach cultivars and a wild relative. Tree Physiol 30:1320–1334.

Stewart-Jones A, Pope TW, Fitzgerald JD, Poppy GM (2008) The effect of ant attendance on the success of rosy apple aphid populations, natural enemy abundance and apple damage in orchards. Agric For Entomol 10:37–43. doi: DOI 10.1111/j.1461-9563.2007.00353.x

Styrsky JD, Eubanks MD (2007) Ecological consequences of interactions between ants and honeydew-producing insects. Proc R Soc B Biol Sci 274:151–164. doi: 10.1098/rspb.2006.3701 10.1007/BF00325261

Suchet C (2010) Ecologie et Evolution des odeurs florales chez Antirrhinum Majus. Université de Toulouse

Sudd J (1967) An introduction to the behaviour of ants. Edwards Arnold, London

Tegelaar K, Hagman M, Glinwood R, et al (2012) Ant-aphid mutualism: The influence of ants on the aphid summer cycle. Oikos 121:61–66. doi: 10.1111/j.1600-0706.2011.19387.x

Turchin P, Kareiva P (1989) Aggregation in Aphis varians: an effective strategy for reducing predation risk. Ecology 70:1008–1016.

Vantaux A, Parmentier T, Billen J, Wenseleers T (2012) Do Lasius niger ants punish low-quality black bean aphid mutualists? Anim Behav 83:257–262. doi: 10.1016/j.anbehav.2011.10.035

Vantaux A, Schillewaert S, Parmentier T, et al (2015) The cost of ant attendance and melezitose secretion in the black bean aphid Aphis fabae. Ecol. Entomol.

Vantaux A, Van den Ende W, Billen J, Wenseleers T (2011) Large interclone differences in melezitose secretion in the facultatively ant-tended black bean aphid Aphis fabae. J Insect Physiol 57:1614–1621. doi: 10.1016/j.jinsphys.2011.08.014

Verheggen F (2008) Production of alarm pheromone in aphids and perception by ants and natural enemies. 197.

Verheggen F, Diez L, Detrain C, Haubruge E (2009a) Aphid-ant mutualism: an outdoor study of the benefits for /i Aphis fabae. Biotechnol Agron Soc Environ 13:235–242.

Verheggen FJ, Diez L, Sablon L, et al (2012) Aphid Alarm Pheromone as a Cue for Ants to Locate Aphid Partners. PLoS One 7:e41841. doi: 10.1371/journal.pone.0041841

Verheggen FJ, Fagel Q, Heuskin S, et al (2007) Electrophysiological and behavioral responses of the multicolored Asian lady beetle, Harmonia axyridis Pallas, to sesquiterpene semiochemicals. J Chem Ecol 33:2148–2155.

Verheggen FJ, Haubruge E, Moraes CM de, Mescher MC (2009b) Social environment influences aphid production of alarm pheromone. Behav Ecol 20:283–288. doi: 10.1093/beheco/arp009

Vilcinskas A (2016) Biology and Ecology of Aphids. CRC Press, Taylor & Francis Group

Völkl W, Woodring J, Fischer M, et al (1999) Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. Oecologia 118:483–491.

Way MJ (1963) Mutualism between ants and honeydew-producing Homoptera. Annu Rev Entomol 8:307–344.

Wheeler W (1926) Ants, their structure, development and behaviour. Columbia University Press, Lancaster

Wilder SM, Barnum TR, Holway DA, et al (2013) Introduced fire ants can exclude native ants from critical mutualist-provided resources. Oecologia 172:197–205. doi: 10.1007/s00442-012-2477-7

Wilder SM, Holway DA, Suarez A V, Eubanks MD (2011) Macronutrient content of plant-based food affects growth of a carnivorous arthropod. Ecology 92:325–332.

Woodring J, Wiedemann R, Volkl W, Hoffmann KH (2007) Oligosaccharide synthesis regulates gut osmolality in the ant-attended aphid Metopeurum fuscoviride but not in the unattended aphid Macrosiphoniella tanacetaria. J Appl Entomol 131:1–7. doi: 10.1111/j.1439-0418.2006.01091.x

Yao I (2014) Costs and constraints in aphid-ant mutualism. Ecol Res 29:383–391. doi: 10.1007/s11284-014-1151-4

Yao I (2012) Ant attendance reduces flight muscle and wing size in the aphid Tuberculatus quercicola. Biol Lett 8:624–627. doi: 10.1098/rsbl.2012.0014 10.1034/j.1600-0706.2000.890101.x)

Yao I, Akimoto S (2001) Ant attendance changes the sugar composition of the honeydew of the drepanosiphid aphid Tuberculatus quercicola. Oecologia 128:36–43.

Yoo HJS, Holway DA (2011) Context-dependence in an ant-aphid mutualism: direct effects of tending intensity on aphid performance. Ecol Entomol 36:450–458. doi: DOI 10.1111/j.1365-2311.2011.01288.x

Zhu J, Cossé AA, Obrycki JJ, et al (1999) Olfactory reactions of the twelve-spotted lady beetle, Coleomegilla maculata and the green lacewing, Chrysoperla carnea to semiochemicals released from their prey and host plant: electroantennogram and behavioral responses. J Chem Ecol 25:1163–1177.

CHAPITRE III

OBJECTIFS DE LA THÈSE

Le contexte général de la présente thèse vient d'être abordé. Il met en évidence à la fois un grand nombre de travaux relatifs au mutualisme fourmis-pucerons et diverses ombres sur l'implication des composés organiques volatils (COVs) dans l'établissement de cette relation particulière entre deux espèces distinctes d'insectes.

Ce travail a comme objectif de mettre en lumière dans quelle mesure les composés sémiochimiques émis dans l'environnement proche des insectes mutualistes étudiés, que ce soit par les insectes eux-mêmes, leurs sécrétions ou par les plantes fréquentées, influencent l'association mutualiste. L'essentiel des travaux portera sur le couple formé par la fourmi noire des jardins, *Lasius niger* L., et le puceron noir de la fève, *Aphis fabae* Scopoli, qui constituent un modèle biologique de référence bien répandu dans son milieu naturel et couramment utilisé pour l'étude du mutualisme fourmis-pucerons. Les divers aspects de cette problématique qui seront abordés peuvent se résumer sous la forme des questions scientifiques suivantes :

- Comment les fourmis s'orientent-elles vers leur partenaire mutualiste ?
    - Quel est le rôle joué par les phéromones émises par une colonie de pucerons ?
    - Quel est le rôle joué par les composés émis par la plante-hôte en réponse à la présence de pucerons ?
    - Quels sont les rôles du miellat dans l'attraction et la reconnaissance à distance ?

    Ces différentes questions seront traitées au cours du chapitre V.
- La présence de fourmis influence-t-elle la sélection d'une plante-hôte par les pucerons ailés, directement ou indirectement ?

  Cette question sera abordée dans le chapitre VI.

CHAPITRE IV

DÉVELOPPEMENTS MÉTHODOLOGIQUES

Les protocoles d'élevages des insectes, des tests biologiques réalisés, de même que des analyses chimiques qui en résultent sont décrits dans les divers articles qui constituent le corps de cette thèse.

Les méthodes d'élevages et les tests biologiques ont été conçus avec pour but de répondre aux questions (objectifs) préalablement énoncées ; et les modes opératoires analytiques (chromatographie en phase gazeuse et GC couplé à la spectrométrie de masse), avant d'être utilisés, ont fait l'objet d'une validation exhaustive. Tous les protocoles touchant à la manipulation d'insectes ont fait l'objet d'une standardisation rigoureuse. A cet égard, et en vue d'éviter tout biais potentiel issu d'un contact direct entre fourmis et pucerons ou d'une exposition prolongée et préalable d'un des partenaires aux composés volatils liés à la présence de l'autre, les élevages des pucerons et fourmis ont pris place dans des locaux séparés

Point critique de toutes analyses, la préparation de l'échantillon conditionne la qualité du résultat final. Elle inclut fréquemment une étape de broyage qui, selon le type d'échantillon et l'objectif à atteindre, peut s'avérer plus ou moins complexe ; pertes, dégradations et contaminations devant être évitées au maximum.

Ainsi, le broyage d'échantillons en vue d'en libérer sans pertes les composés volatils, la nécessité de conditions stériles, la présence de composés potentiellement sensibles à l'air, et le traitement d'échantillons de très petite taille constituent quelques-unes des contraintes où le broyage d'un échantillon peut s'avérer complexe. Au cours de ce travail, de telles situations se sont fréquemment présentées. La plupart des systèmes couramment disponibles en laboratoire n'offrent pas de solutions parfaitement adéquates, et les appareillages plus spécifiques, quand ils existent, sont souvent coûteux.

Dans ce chapitre, une méthode simple, efficace, automatisable et réalisable avec du matériel de base sera décrite. Elle sera utilisée à diverses reprises au cours du travail de recherche. Elle a, en outre, été appliquée avec succès à d'autres problématiques (Fischer *et al.* 2012; Fassotte *et al.* 2014; Censier *et al.* 2014).

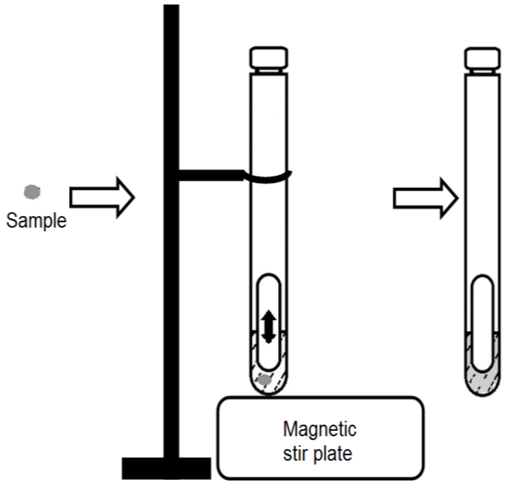
SIMPLE AND AUTOMATIC CLOSED GRINDING AND EXTRACTION SYSTEM

Christophe Y. Fischer, Georges C. Lognay

Laboratory of Analytical Chemistry, University of Liege, Gembloux Agro-Bio Tech, Passage des Déportés 2, B-5030 Gembloux (Belgium)

Abstract - This article describes a very simple automatic and closed grinding system for small samples using only common laboratory equipment, particularly useful for air sensitive samples or volatile compounds analyses.

Keywords: laboratory equipment, bioanalytical chemistry, analytical chemistry, general public



Citation: *Fischer CY, Lognay GC (2012) Simple and Automatic Closed Grinding and Extraction System. J Chem Educ 89:1611–1612. doi: 10.1021/ed2007907*

Introduction

A great number of chemical analyses start by grinding and extracting the sample. This apparently elementary step can become quite tricky when having to deal with small quantities of air sensitive or volatile organic compounds (VOC) emitting materials. Most common methods involve manual mortar and pestle or bladed devices. None of those are easy to get really airtight. Moreover, the first one cannot be automated, and the second one is often difficult to clean, which could lead to cross contamination and analyte losses. Furthermore, dedicated grinding systems could sometimes be quite costly.

This article describes a very simple protocol to easily solve this problem using only common laboratory equipment.

This protocol has been successfully applied to the lossless extraction of insects' secondary metabolites (Fischer *et al.* 2012) and to plant VOC sampling, and could be used for a large number of applications involving the crushing or grinding of VOC emitting or air sensitive samples.

Material

This method requires only a narrow tightly closing container (ex.: Sovirel-type tube), a stand with a holder, a magnetic stir bar fitting the inside of the tube and a magnetic stir plate.

Any kind of narrow strong walled glassware could be used, but best results were obtained with round bottomed glass tubes, which best fit the end of stir bars, and where sample particles remain in the center of the tube, where the stir bar action is the strongest. Tubes should be high enough to let the stir bar move freely. Maximal suitable glassware size is defined by the couple stir bar / stir plate. Optimal stir bar should fit the inside of the tube, while not too tightly to avoid being stuck with sample particles. Too large glassware will require large stir bars, which would become too strong for the stir plate and would slow it down too much, or even block it depending on the stir plate strength. For hard samples, a tip of Pasteur pipette (or any fine piece of glass which could be easily broken and reduced to a fine powder) can be added to improve the grinding.

Method

Place the sample in the container, if needed with a small quantity of extraction solvent (total volume should not exceed the third of the container, which would too much slow down the stir bar). Add the magnetic stir bar and the tip of a Pasteur pipette for the fine grinding of hard samples. Position this system on the magnetic plate and turn it on (Figure 1). Avoid any space or particles (sand, glass fragments…) between plate and tubes to prevent them from breaking. When the container is at the right place on the plate, with the right agitation frequency (both determined by trial and error), the magnetic stir bar will alternately be propelled upwards and attracted downwards because of the alternating magnetic field, acting like an automatic pestle. Quickly powdered, the Pasteur pipette tip will enhance this system efficiency by its abrasive properties. This system is left to extract until the right grinding is obtained. Using a 45mm stir bar with a IKA labortechnik RCT Basic magnetic plate (Filter Service, Eupen, Belgium), fine grinding of small insects is completed in less than two minutes. Figure 2 shows a ladybird beetle after a two minutes grinding in methanol. The crushing of plants in order to analyze VOC is achieved in a matter of seconds.

Being completely closed, this system avoids any analyte losses. For air sensitive compounds, the container should be filled with nitrogen prior to extraction. This system is also suitable for VOC analyses by using septum caps. For passive solid phase micro-extraction (SPME) sampling, the fiber is simply exposed by piercing the septum. For active sampling, incoming air has to be cleaned, which can be done using the setup presented in Figure 3.

Acknowledgment

Christophe Fischer is financially supported by the *Fond pour la Formation à la Recherche dans l'Industrie et l'Agriculture* (FRIA, Belgium).

Supporting information

Two videos showing the described system in function are available in annexed files. Annex 1 shows the grinding in solvent under nitrogen atmosphere of a ladybird in order to study its secondary metabolites. Annex 2 shows the sampling of crushed *Arabidopsis* *thaliana* volatile compounds. This material is available via the Internet at http://pubs.acs.org.

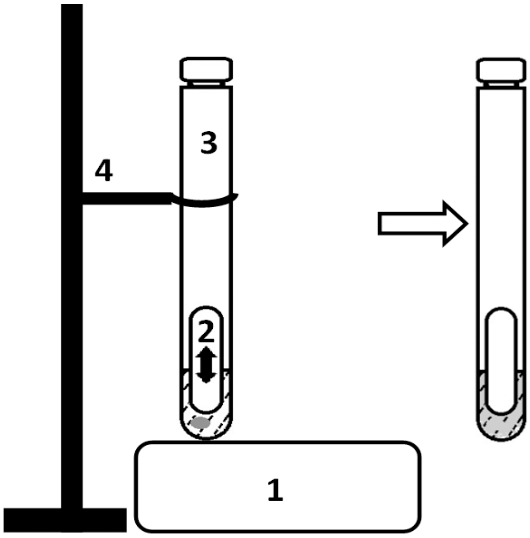


Figure 1. : Extraction system. (1) magnetic stir plate, (2) magnetic stir bar, (3) Sovirel-type tube with sample and extraction solvent if needed, (4) holder

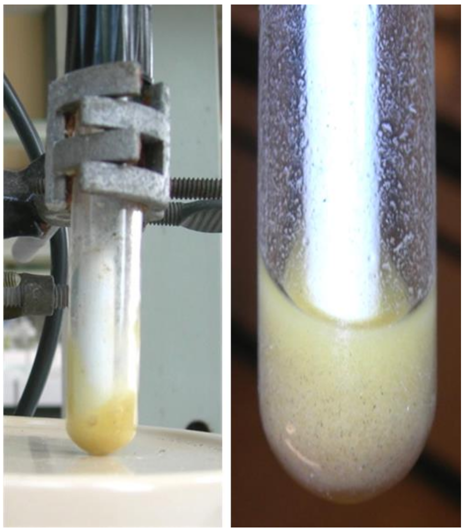


Figure 2. : Ladybird beetle after a two minutes grinding in methanol

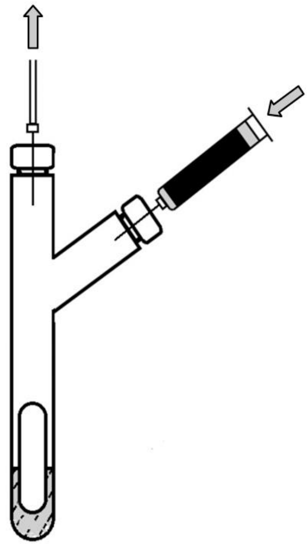


Figure 3. : Adapted system for active VOCs sampling. Incoming air is cleaned for example on activated charcoal maintained in a glass syringe by two glass wool plugs. In this case, caps with septa are used. Arrows indicate the air flow in this system.

References

Fischer CY, Laurent P, Heuskin S, et al (2012) Fast-GC quantification of harmonine, the major defense alkaloid of the multicolored Asian ladybird Harmonia axyridis (Pallas). Am J Anal Chem 3:67–75.

CHAPITRE V

RECHERCHE D’UN PARTENAIRE MUTUALISTE PAR LA FOURMI

Préalablement à toute possibilité de mutualisme, les partenaires mutualistes doivent se rencontrer. Malgré l'évidence de ce fait, les facteurs sémiochimiques influençant la rencontre entre une fourmi exploratrice et une colonie de pucerons restaient largement méconnus. Ce chapitre portera sur le rôle des composés volatils dans l'attraction des fourmis par les pucerons, et sera divisé en trois sous-questions, qui seront traitées sous forme de trois publications :

- Quel est le rôle du E-*β*-farnésène, principal composé phéromonal régulièrement émis par le puceron, dans l'attraction des fourmis ?
- Quels sont les effets et l'origine des composés induits par la présence de pucerons, c'est-à-dire émis par la plante attaquée et par le miellat produit par l'insecte, dans l'attraction des fourmis ?
- Ces sémiochimiques permettraient-ils aux fourmis de différencier à distance diverses espèces de pucerons ?

APHID ALARM PHEROMONE AS A CUE FOR ANTS TO LOCATE APHID PARTNERS

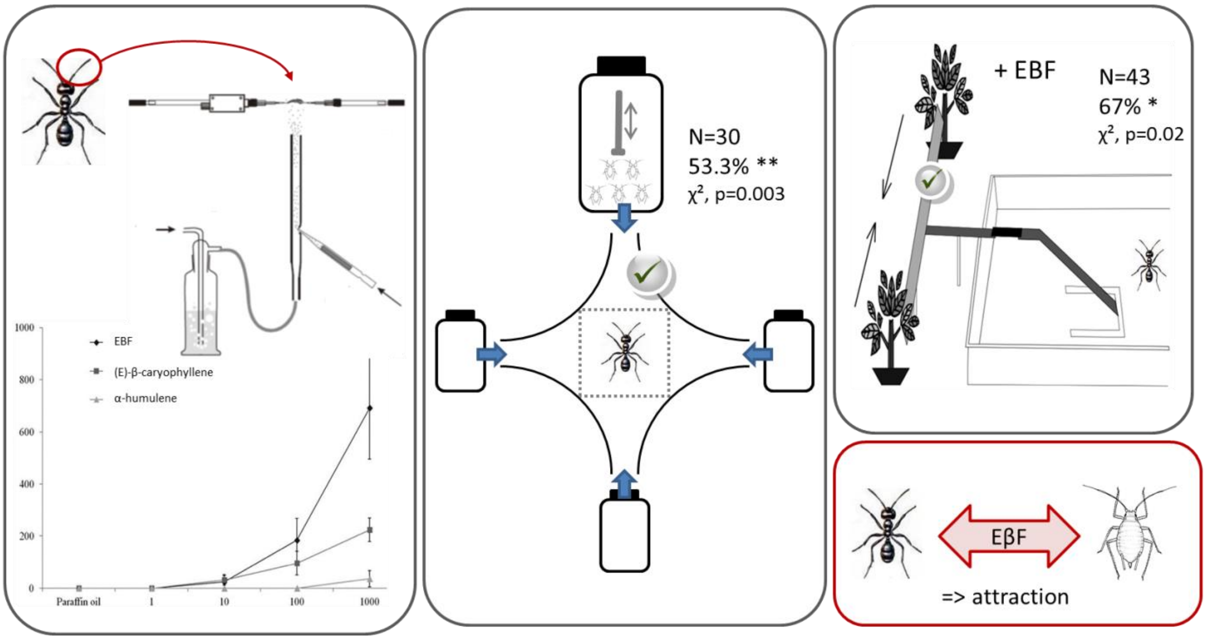
François J. Verheggen[1]*, Lise Diez[2], Ludovic Sablon[1], Christophe Fischer[3], Stefan Bartram[4], Eric Haubruge[1], Claire Detrain[2]

1 Department of Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium

2 Department of Social Ecology, University of Brussels, Brussels, Belgium

3 Department of Analytical Chemistry, Gembloux Agro-Bio Tech, University of Liege, Gembloux, Belgium

4 Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena, Germany



Graphical abstract – Aphid alarm pheromone as a cue for ants to locate aphid partners

Abstract: The mutualistic relationships that occur between myrmecophilous aphids and ants are based on the rich food supply that honeydew represents for ants and on the protection they provide against aphid natural enemies. While aphid predators and parasitoids actively forage for oviposition sites by using aphid semiochemicals, scouts of aphid-tending ant species would also benefit from locating honeydew resources by orienting toward aphid pheromone sources. The present study aims to provide additional information on the use of *Aphis fabae* alarm pheromone, i.e. (*E*)-β-farnesene (EBF), by ant scouts. The perception and behavioral impact of EBF on *Lasius niger* were investigated using electroantennography and two bio-assays measuring their attraction and orientation towards aphid semiochemicals. Pronounced electrical depolarizations were observed from *L. niger* scout antennae to stimulations of *A. fabae* alarm pheromone, while other sesquiterpenes elicited weak or no responses. *L. niger* scouts were significantly attracted toward EBF in a four-arm olfactometer, as well as in an two-choice bioassay. These laboratory results suggest for the first time that low amounts of aphid alarm pheromone can be used by *L. niger* scouts as a cue indicating the presence of aphid colonies and could therefore mediate the aphid-ant partnership in the field.

Citation: *Verheggen FJ, Diez L, Sablon L, Fischer C, Bartram S, Haubruge E and Detrain C (2012) Aphid Alarm Pheromone as a Cue for Ants to Locate Aphid Partners. PLoS ONE 7(8): e41841. doi:10.1371/journal.pone.0041841*

Introduction

Aphids (Hemiptera, Aphididae) and ants (Hymenoptera, Formicidae) are the protagonists of one of the most studied model of mutualistic relationships in the animal kingdom: the first ones produce a carbohydrate-rich excretion named honeydew, which is collected by some ant species who provide aphids in return with protection and hygiene (Way 1963).

The communication between both partners was thought to be essentially tactile, as ants palpate aphids' abdomen using alternatively their two antennae to stimulate the ejection of honeydew droplets. But the interactions between ants and aphids are also chemically mediated. Nault and co-authors (Nault *et al.* 1976) have indeed demonstrated *Formica subsericea* ability to react behaviorally to the main component of the alarm pheromone of most aphidinae species. When *F. subsericea* were attending aphids and suddenly exposed to huge amounts of (*E*)- β -farnesene (EBF), they extended their antennae and opened their mandibles being prepared for attacking potential aphid enemies. Besides, more recent studies have demonstrated that ants detect specific blends of cuticular hydrocarbons on aphids' body what allows them to discriminate myrmecophilous aphids from potential prey (Lohman *et al.* 2006). However, nothing is known about the possible chemical detection of aphids by ants from a distance, the first encounter between both insect species being usually assumed to occur by chance.

Several laboratory studies have suggested that aphid natural enemies, including ladybeetles, hoverflies and chrysopids, may be able to detect EBF and use it as a kairomonal substance to locate their host or prey (Micha and Wyss 1996; Zhu *et al.* 1999; Abassi *et al.* 2000; Verheggen *et al.* 2007; Verheggen *et al.* 2008a; Verheggen *et al.* 2009b). Here, we studied whether ant scouts, i.e. workers mainly involved in exploration and recruitment, are also able to locate aphids by detecting this sesquiterpene at the lower levels that are usually emitted by unthreatened aphid colonies outside any alarm context. The perception and behavioral impact of EBF on *Lasius niger* were investigated using electro-antennography and two bio-assays measuring their attraction and orientation towards aphid semiochemicals.

Materials and Methods

Ants and Aphids

Queenless *Lasius niger* L. colonies (500 individuals) were collected in Brussels in April 2007 and placed in plastic containers (35x25x8 cm) whose edges were covered with polytetrafluoroethylen to prevent them from escaping. Test tubes covered with a red transparent foil were disposed as laboratory rearing nests. Sucrose solutions (1 M), dead arthropods (cockroaches, aphids and spiders) and water filled test tubes were provided and renewed every two days. The colonies were kept in an environmentally controlled room (L16:D8, humidity 65±5%, and 23±1°C). The black bean aphids, *Aphis fabae* Scopoli, were mass reared on broad beans (*Vicia faba* L.) grown in 10 cm^3 plastic pots filled with a mix of perlite and vermiculite (1:1) and placed in similar conditions as above.

Electroantennography

L. niger scout antenna was carefully excised from the head. Because of the important background noise registered from the ant antenna, the scape was removed to improve electrical contact and subsequently decrease background noise. The antenna was mounted and stimulated as described in our previous experiments on the perception of aphid alarm pheromone by beetle and fly antennae (Verheggen *et al.* 2007; Verheggen *et al.* 2008a). Paraffin oil was used to make four EBF solutions with concentrations ranging from 0.1 g/l to 100 g/l (by 10x increments). Stimulation with semiochemical-free paraffin oil was carried out as a negative control before and after the stimulations with the four EBF solutions cited above. Thirty seconds elapsed between successive stimulations. Preliminary results indicate that this length of time was adequate to allow the insect recover its full reactivity to stimuli. EBF was synthesized from farnesol with a chemical purity of 98% (determined by GC). In order to compare the scout antenna sensibility to EBF with other sesquiterpenes (but not associated to aphids), (*E*)-caryophyllene and a-humulene, both purchased from Sigma-Aldrich (Chemie Gmbh, Steinheim, Germany) were also tested following the same procedure as above. A total of 15 different antennae were tested: five per chemical. Each antenna was tested with the 5 concentrations of one single chemical (paraffin oil control and the four doses in increasing order: 1, 10, 100 and 1000 mg).

Four-arm Olfactometer Assays

The four-arm olfactometer was similar to that previously described by Verheggen *et al*. (Verheggen *et al.* 2007) and was adapted to be connected to a *L. niger* colony. It was constructed entirely of Teflon® and was closed with a removable glass roof, both cleaned with *n*-hexane between each tested ant. Charcoal-filtered air was pushed in each of the four olfactometer arms through Teflon® tubing, and adjusted to 100 ml/min for each arm with a digital flowmeter. A pump ventilated the walking arena by removing air from the centre at 400 ml/min. A *L. niger* colony was placed under the olfactometer and a Teflon® tube allowed scouts to climb up to the walking arena. A "T" glass piece allowed the connection of the plastic tube to the olfactometer, and at the same time the aspiration of the outgoing air. This piece also allowed to close the access to the olfactometer and thus controlled the entrance of only one scout per replicate. A 0.5 l glass chamber was connected to one of the four olfactometer arms, and was used to introduce five unwinged *A. fabae* adults, that were rapidly crushed inside the glass chamber using a small glass pestle left inside the chamber (as a natural source of EBF). According to Pickett & Griffiths (Pickett and Griffiths 1980) and Francis *et al*. (Francis *et al.* 2005), the volatiles released by crushed *A. fabae* consist exclusively of EBF. Preliminary volatile analysis experiments allowed us to approximate the amount of EBF released by five crushed *A. fabae*. Five *A. fabae* individuals were quickly crushed in *n*-hexane and the supernatant was injected in a gas chromatograph. We found an average amount of 50.9 ng of EBF, which is similar to what a quiet non-preyed *M. persicae* colony made of about 75 individuals release (Almohamad *et al.* 2008). The glass chamber was randomly connected to one of the four arms of the olfactometer. The olfactometer was divided into one central 10 cm squared area, and four other areas related to the four odor sources. The observations were conducted for 3 min, starting when the scout entered the walking arena. The choice of the tested scout was determined by (a) the first area it entered and (b) the time spent in each of the four areas. The behavioral observations were conducted on 30 ant scouts in a laboratory at 22±1°C and under uniform lighting.

Two-choice Bioassay

The setup (Fig. 1) was made of aluminium and consisted in different parts that were explored by single tested ants: a single ant scout was allowed to climb the access ramp (length 35 cm, width 1 cm) which was placed near the nest entrance with a 45° incline. (2) A 3-cm section of this ramp was manually removed to avoid additional scouts to reach the "T"setup. (3) The tested scout was then reaching the "T"setup, which was composed of two branches disposed at 90° from the access ramp, and both of a length of 25 cm and a width of 1 cm. Each branch led the observed ant scout to one of the two tested plants. A small space (1 cm) was left between each plant and the end of the setup branches to ensure that ants could not climb upon leaves and stems (4). A rubber septum containing pure EBF was placed alternatively (with similar number of replicates being conducted on both sides) on one of the two plants and switched after each observed ant scout. One ventilator was placed behind each plant to ensure an air flow of 0.6±0.1 m/s, going from the plant to the bioassay setup. The "T" setup was divided into different sections: the middle part of the "T" aluminum (8 cm length) was considered as an area of no-choice. The last 1.5 cm of the "T" foraging branches were considered as areas where the final choice was made by the ants which were removed after having reached one of these sections. The time spent by each ant scout in both "T" arms, the final choice and the number of U-turns were recorded. The walking speed has been calculated by dividing the time spent in one of the two sections of the bioassay by the length of the section. Ants that changed direction (i.e. side of the olfactometer) during the test were not taken into account for this calculation. The setup was surrounded by black plastic sheets to avoid visual bias and disturbances, and was placed under uniform light provided by three neon tubes. Three different ant colonies were tested and results were pooled, after having checked the absence of bias. A total of 43 ant scouts were observed.

Statistical Analyses

To compare EAG responses for the three tested chemicals (EBF; (*E*)-caryophyllene; α-humulene) at the 5 different doses (control; 1 mg; 10 mg; 100 mg; 1000 mg), a three-way ANOVA was conducted with factors being "chemicals" (systematic factor), "doses"(systematic factor) and "antennae"(random factor). Because every doses were tested on every antennae, and because only one chemical was tested per antenna, we used a partially hierarchized model: the factor "doses" is crossed with the factor "chemicals" and with the factor "antennae", while the factor "antennae "is hierarchized with the factor "chemical". Observed frequencies related to the final choice of *L. niger* scouts in olfactometer assays (four-arm and two-choice bioassays) were compared to corresponding theoretical frequencies by using a x2 goodness-of-fit test. Paired t-test was conducted to compare, for each ant scout, the difference between the time spent in the branch leading to the EBF treated plant and the time spent in the branch leading to the control plant. ANOVA were conducted to compare the mean durations spent in the different branches of both bioassays. Finally, Fisher's exact tests were conducted to compare proportions of ants initiating specific types of behavior in the bioassays. All these tests were conducted with MINITAB v15 (State College, Pennsylvania, USA).

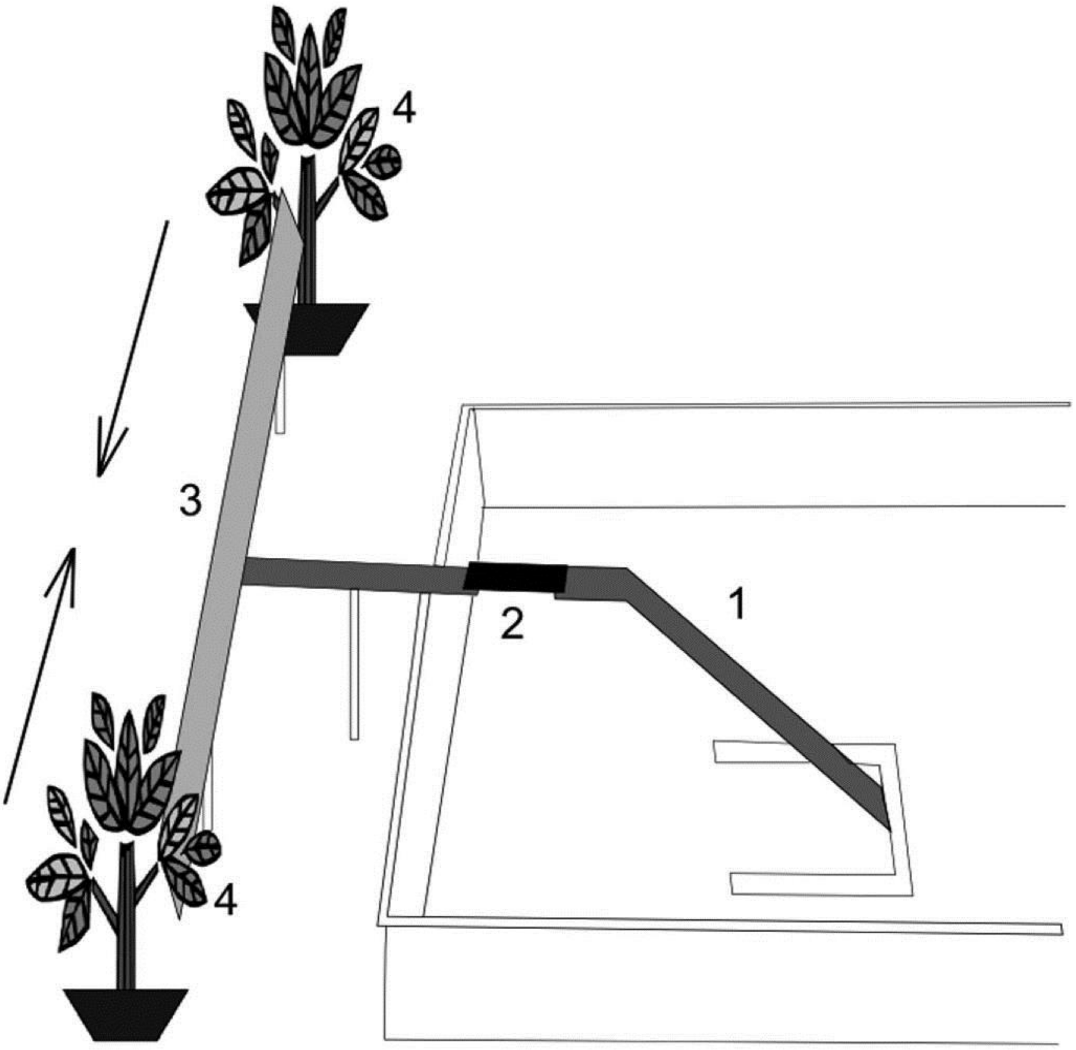


Figure 1. Experimental setup used to study foraging behaviour of individual scouts. (1) Access ramp; (2) movable section of the bridge;(3) ''T'' foraging arena, (4) Uninfested Faba beans. Arows indicate directions of the airflow.

Results

Electroantennography

The highest dose of EBF elicited EAG responses of -0.692±0.197 mV (mean ±SE). Two additional sesquiterpenes were also tested and both (E)-caryophyllene (-0.224±0.045 mV) and a-humulene (-0.036±0.031 mV) elicited weak electrical depolarization from *L. niger* antennae (Fig. 2.). A three-way ANOVA was conducted to compare EAG responses for the three tested chemicals (EBF; (E)-caryophyllene; a-humulene) at the 5 different doses (control; 1 mg; 10 mg; 100 mg; 1000 mg). The electrical responses recorded differed statistically for all three semiochemicals tested (ANOVA, F2,12 = 11.42, P = 0.002). A positive dose–response relationship in EAG was also observed (ANOVA, F4,48 = 15.68, P<0.001). The three-way ANOVA highlighted an interaction relationship between the two systematic factors, namely the chemicals and the doses (ANOVA, F8,48 = 6.07, P<0.001). We have therefore conducted a two-way ANOVA for each tested chemical. A positive dose–response relationship in EAG was recorded to EBF (F4,16 = 9.09, P<0.001) and to (E)-caryophyllene (F4,16 = 14.68, P<0.001), but not to a-humulene (F4,16 = 1.33, P = 0.302). When conducting a two-way ANOVA separating each tested doses, we found that, at the highest tested dose, the recorded electrical responses differed statistically between the three semiochemicals tested (ANOVA, F2,8 = 9.30, P = 0.008), with EBF eliciting the highest electrical response.

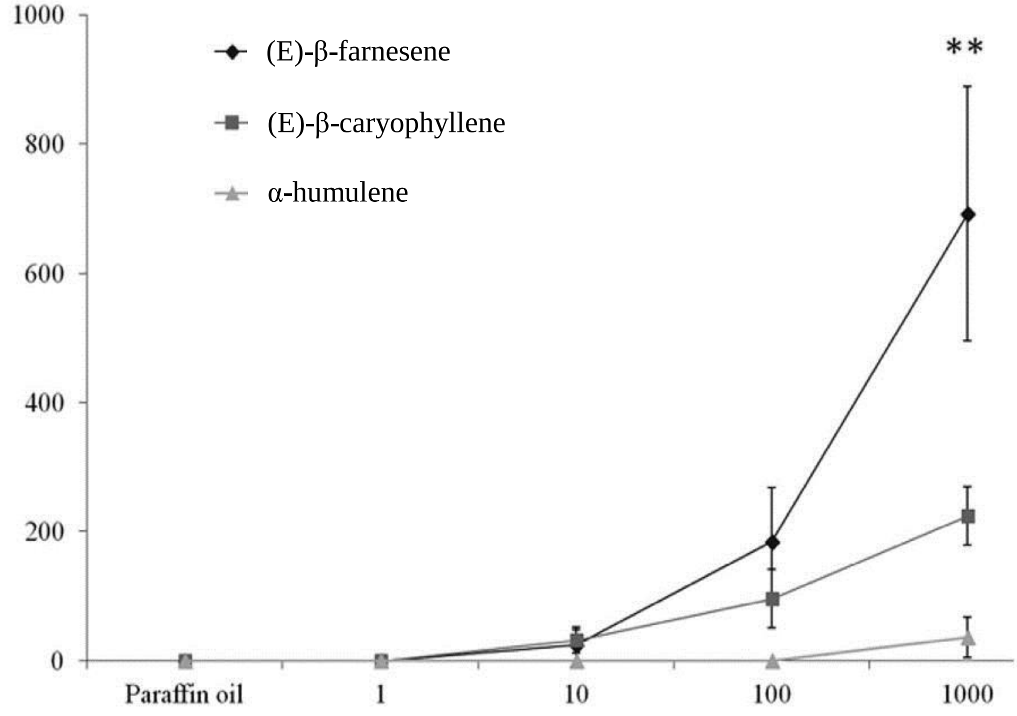


Figure 2. Effect of (*E*)-β-farnesene (aphid alarm pheromone), (*E*)-β-caryophyllene and α-humulene on the antennal responses (±SE) of *Lasius niger* scouts (n = 5). **indicate significant EAG responses at P,0.01.

Four-arm Olfactometer Assays

According to the first area visited, 53.3% of the scouts were first attracted to the EBF source. This visitation rate is significantly higher than expected from a random orientation (25% in the case of a random choice) ($x^2 = 9.63$, *P* = 0.003, n = 30). They also spent most of their time in the arena connected to the EBF source, as the tested scouts spent 42.7±6.2% of the observation time (time spent in the neutral area deducted) in the EBF arm of the olfactometer ($F_{3,116} = 3.02$, $P = 0.033$, $n = 30$).

Two-choice Bioassay

Ant scouts preferentially orientated towards the branch leading to the EBF treated plant (67%) rather than to the branch leading to the control non treated plant (33%) (n = 43, $x^2 = 5.23$, P = 0.02). For each ant scout, the time spent on the branch leading to the EBF treated plant (mean = 8.28 sec, n = 43) was on average significantly higher than the time spent on the branch leading to the control plant (mean = 3.59 sec, n = 43) (Paired t-test, T-value = 2.97, P = 0.005). Before ant scouts orient themselves toward one side or the other, we also recorded the time spent in the neutral area as an estimate of their difficulty to make a choice, this time being assumed to be shorter for ants being attracted by the EBF treated side. But, no difference was observed between the mean time spent in the neutral area by ant scouts choosing the EBF side (3.8±0.6 s) and that for ants choosing the untreated side (3.8±0.8 s) (ANOVA, F1,41 = 0.01, P = 0.978). On average, the walking speed were similar for ants orientating to the control (1.74±0.12 cm/s) and the EBF side (1.96±0.23 cm/s) of the bioassay (ANOVA, F1,41 = 0.94, P = 0.338). Finally, we noted the number of ant scouts "changing their mind"– i.e. first walking over one branch, then making U-turns and finally choosing the other side of the setup. Over the ants having finally chosen the EBF side, only 10% of scouts had initially strolled over the untreated zone. Indeed, a majority (90%) of scouts orienting themselves towards EBF treated side made this clear-cut choice from the start of the experiment. As regards ant scouts having finally chosen the untreated side, we found out a higher, but not statistically significant, percentage (29%) of "hesitating "individuals that first initiated a short movement towards the EBF treated plant before changing side (Fisher's exact test, P = 0.190).

Discussion

Like most aphid natural enemies that have evolved to adapt their olfactory system to the perception of aphid-related volatile chemicals and subsequently locate their prey, ants would have advantage to perceive aphid odorant cues, which would increase their chance to establish a mutualistic relationship. Our results demonstrate that *L. niger* have olfactory receptors perceiving *A. fabae* alarm pheromone, as shown by the positive dose–response relationship in EAG to EBF. The highest tested EBF dose elicited EAG responses of -0.692±0.197 mV (mean ±SE) statistically higher than the paraffin oil control (Fig. 1.). While using EBF at the same dose, and with similar equipment and method, Verheggen *et al.* (2007, 2008) obtained EAG responses twice lower with the predatory hoverfly *Episyrphus balteatus* (Diptera, Syrphidae), and three times lower with the Asian lady beetle, *Harmonia axyridis* (Coleoptera, Coccinellidae). Moreover, other sesquiterpenes a-humulene and (*E*)-caryophyllene did not elicit pronounced electrical depolarizations. The olfactory system of foraging ant workers therefore seems to be sensitive and adapted for the perception of aphid alarm pheromone. We also showed that *L. niger* scouts detect (*E*)-caryophyllene, as low electrical responses were recorded from scouts antennae. As observed for aphid natural enemies (Abassi *et al.* 2000) this might serve ants to make the distinction between pure EBF emitted by aphids and EBF from some plant species that is emitted along with other sesquiterpenes like (*E*)-caryophyllene.

Aphid alarm pheromone is known to elicit agonistic behaviour – i.e. raising of antennae and opening of mandibles – among *Formica subsericea* ant species (Nault *et al.* 1976). A field study, where high doses of synthetic alarm pheromone were applied on pea aphid *Acyrthosi-phon pisum* colonies, has reported an increase in the number of predating *Lasius niger* ants in the treated aphid colonies (Outreman *et al.* 2010), suggesting that alarm signaling in aphids is associated with the ecological cost of attracting additional natural enemies. Presentation of a filter paper impregnated with large amounts of pure EBF also induced typical alarm and defensive behavior among *Lasius niger* ants (pers. obs.). That low EBF levels – *i.e.* the background level emitted by quiet and non-preyed aphid colonies – could be perceived by ant scouts, looking for food resources, and thus be used as a cue to locate their aphid partner has never been demonstrated earlier. Single ant scouts were clearly attracted by EBF in the four-arm olfactometer. This has been observed also in our two-choice bioassay. In both cases, none of the observed ant scouts exhibited agressive behaviour like that observed by Nault *et al*. (1976). Furthermore, their walking speed (1.7–2.0 cm/s) were similar to that previously reported for *L. niger* scouts foraging for food (1.6 to 2.1 cm/s) (Mailleux *et al.* 2000). This suggests that the conditions of the bioassay (i.e. exposure to low and constant amounts of EBF) have led to attraction rather than an alarm or defensive behaviour. The fact that EBF induces quite different behavioural responses among ant scouts depending on the perceived amounts might have strong ecological implications, and may explain the increase in ant predation behavior observed in pea aphid colonies where additional amounts of alarm pheromone were added (Outreman *et al.* 2010). Alarm pheromone is emitted either in case of attacks by natural enemies but is also released, at very low doses, from non-attacked *M. persicae* colonies (Almohamad *et al.* 2008). When aphids are endangered, the emission of high EBF levels triggers aggressive behaviours among ants and thus speeds up their chasing and killing of predators/parasitoids. Many ant species, including *Lasius niger*, are also known to switch continuously from a "breeder" to a "predator" behavior according to aphid colony size (Sakata 1995). Indeed, the increased aphid density per ant led to an increase in the rate of predation(Sakata 1995). The constant released amount of alarm pheromone by anon-preyed aphid colony informs natural enemies about the aphid colony density (Almohamad *et al.* 2008). That the amount of volatile cues also used by ants to evaluate the aphid density of a colony still remains to be experimentally investigated. One may however hypothesize that, at high levels of emission, EBF could facilitate the shift from a "breeder" behavior of tending ants to a "predator" behavior, when the aphid colony gets crowded EBF is used as unique component of the alarm pheromone in most aphid species, including unattended ones (Francis *et al.* 2005).

If EBF leads mostly to aphid colonies, and sounds like reliable semiochemical for aphid presence, one could consider its perception by ants as either an indicator of a mutualism opportunity, or a source of food. Regarding aphids, they would have strong advantage to emit low amounts of semiochemicals to attract ants at the first steps of this mutual relationship. Once this first contact established, ants will assess the profitability of this aphid colony such as the quality, the amount or the renewal rate of produced honeydew (Mailleux *et al.* 2000; Mailleux *et al.* 2003a; Detrain *et al.* 2010). Depending upon this food profitability, a more or less intense trail will be laid by the ant: this trail will recruit nestmates, guide them to already discovered aphid colonies and acts as the main driver for collective selection and exploitation of this food resource (Hölldobler and Wilson 1990; Detrain *et al.* 2010).

Within aphid-ant mutualism, aphid semiochemicals, including the aphid alarm pheromone, could act as synomones, being beneficial for the releasers (aphids), that will attract their bodyguards, and beneficial for the receivers (ant scouts), that will likely encounter a food source. This assumption should however be confirmed by performing field assays demonstrating that in natural conditions, emissions of EBF attract ant scouts.

Because the aphid alarm pheromone is not the only semiochemical to be released by an aphid colony, one should also evaluate the biological activity of other aphid-related volatile chemicals, including those released by the aphid honeydew (Leroy *et al.* 2011a), in the establishement of an aphid-ant partnership.

Acknowledgment

Dr. Detrain is senior research assistant at Belgian foundation for scientific research (FNRS). The authors work has been funded by a FNRS (*Fonds National de la Recherche Scientifique*) grant (n°2.4600.09). Dr. Sablon is financially supported by a CURAGx PhD grant. Dr. Fischer and Dr. Diez are financially supported by F.R.I.A. grants. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Abassi SAL, Birkett MA, Pettersson J, et al (2000) Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. J Chem Ecol 26:1765–1771. doi: 10.1023/A:1005555300476

Almohamad R, Verheggen FJ, Francis F, et al (2008) Emission of alarm pheromone by non-preyed aphid colonies. J Appl Entomol 132:601–604. doi: 10.1111/j.1439-0418.2008.01309.x

Detrain C, Verheggen FJ, Diez L, et al (2010) Aphid-ant mutualism: how honeydew sugars influence the behaviour of ant scouts. Physiol Entomol 35:168–174.

Francis F, Vandermoten S, Verheggen F, et al (2005) Is the (E)-β-farnesene only volatile terpenoid in aphids? J Appl Entomol 129:6–11.

Hölldobler B, Wilson EO (1990) The Ants. Springer-Verlag GmbH

Leroy PD, Sabri A, Heuskin S, et al (2011) Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. Nat Commun 2:7. doi: Artn 348 Doi 10.1038/Ncomms1347

Lohman DJ, Liao Q, Pierce NE (2006) Convergence of chemical mimicry in a guild of aphid predators. Ecol Entomol 31:41–51. doi: 10.1111/j.0307-6946.2006.00758.x

Mailleux AC, Deneubourg JL, Detrain C (2000) How do ants assess food volume? Anim Behav 59:1061–1069.

Mailleux AC, Deneubourg JL, Detrain C (2003) Regulation of ants’ foraging to resource productivity. Proc R Soc London Ser B-Biological Sci 270:1609–1616. doi: DOI 10.1098/rspb.2003.2398

Micha SG, Wyss U (1996) Aphid alarm pheromone (E)-β-farnesene: a host finding kairomone for the aphid primary parasitoid Aphidius uzbekistanicus (Hymenoptera: Aphidiinae). Chemoecology 7:132–139.

Nault LR, Montgomery ME, Bowers WS (1976) Ant-aphid association: role of aphid alarm pheromone. Sci USA 192:1349–1351.

Outreman Y, Kunert G, Simon J-C, Weisser W (2010) Ecological Costs of Alarm Signalling in Aphids. In: Kindlmann P, Dixon AFG, Michaud JP (eds) Aphid Biodiversity under Environmental Change SE - 10. Springer Netherlands, pp 171–181

Pickett JA, Griffiths DC (1980) Composition of aphid alarm pheromones. J Chem Ecol 6:349–360.

Sakata H (1995) Density-dependent predation of the ant Lasius niger (Hymenoptera: Formicidae) on two attended aphids Lachnus tropicalis and Myzocallis kuricola (Homoptera: Aphididae). Res Popul Ecol (Kyoto) 37:159–164.

Verheggen FJ, Arnaud L, Bartram S, et al (2008) Aphid and plant volatiles induce oviposition in an aphidophagous hoverfly. J. Chem. Ecol. 34:301–307.

Verheggen FJ, Fagel Q, Heuskin S, et al (2007) Electrophysiological and behavioral responses of the multicolored Asian lady beetle, Harmonia axyridis Pallas, to sesquiterpene semiochemicals. J Chem Ecol 33:2148–2155.

Verheggen FJ, Haubruge E, Moraes CM de, Mescher MC (2009) Social environment influences aphid production of alarm pheromone. Behav Ecol 20:283–288. doi: 10.1093/beheco/arp009

Way MJ (1963) Mutualism between ants and honeydew-producing Homoptera. Annu Rev Entomol 8:307–344.

Zhu J, Cossé AA, Obrycki JJ, et al (1999) Olfactory reactions of the twelve-spotted lady beetle, Coleomegilla maculata and the green lacewing, Chrysoperla carnea to semiochemicals released from their prey and host plant: electroantennogram and behavioral responses. J Chem Ecol 25:1163–1177.

BACTERIA MAY ENHANCE SPECIES ASSOCIATION IN AN ANT-APHID MUTUALISTIC RELATIONSHIP

Christophe Y. Fischer[1*], Georges C. Lognay[1], Claire Detrain[2], Martin Heil[3], Alina Grigorescu[4], Ahmed Sabri[4], Philippe Thonart[4], Eric Haubruge[5], François J. Verheggen[5]

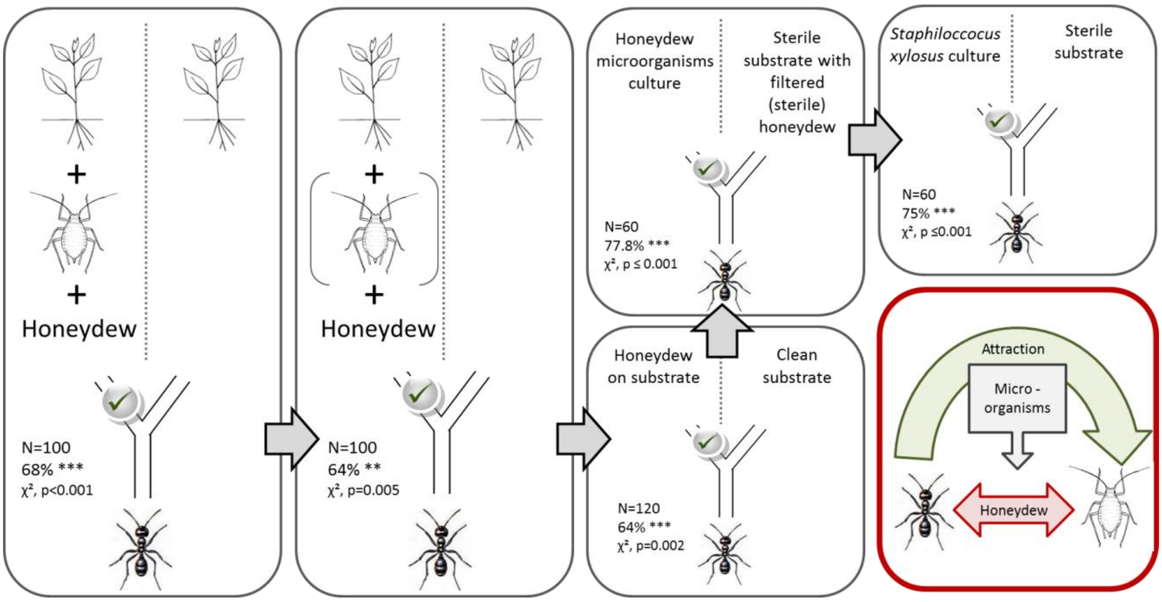
1 Laboratory of Analytical Chemistry, University of Liege, Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium.

2 Unit of Social Ecology, Université Libre de Bruxelles, CP231,50 avenue F. Roosevelt, B-1050 Brussels, Belgium.

3 Departamento de Ingenieria Genética, Centro de Investigacion y de Estudios Avanzados del Instituto Politécnico Nacional, Irapuato, Km. 9.6 Libramiento Norte, CP 36821, Irapuato, Guanajuato, México.

4 Walloon Center of Industrial Biology, University of Liege, B40, 4000 Sart-Tilman, Belgium.

5 Department of Functional and Evolutionary Entomology, University of Liege, Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium.



Graphical Abstract – Bacteria aid partner association in an ant aphid mutualism

Abstract : The mutualistic relationships between certain ant and aphid species are well known, the primary benefits being protection for the aphids and carbohydrate-rich honeydew for the ants. Questions remain, however, as to the exact semiochemical factors that establish and maintain such relationships. In this study we used a series of treatments and associated controls placed at the end of a two-way olfactometer to determine the degree of attractiveness of a complete plant-aphid-honeydew system as well as individual components of that system. Both the olfactometer branch selected by the black garden ant (*Lasius niger*), and the linear speed with which ants moved through the device, were measured. Study results showed that ants were attracted not just to the complete plant system and the honeydew itself, but also to the microbial flora in the absence of plant or honeydew, and specifically to a bacterium from the black bean aphid (*Aphis fabae*) honeydew, *Staphylococcus xylosus*. This bacterium produces a blend of semiochemicals that attract the ant scouts. This information suggests the presence of a naturally-occurring, reliable biotic cue for detection of potential aphid partners. This would have to be confirmed in natural conditions by further field experiments.

Rather than being opportunistic species that coincidentally colonize a sugar-rich environment, microorganisms living in aphid honeydew may be able to alter emissions of volatile organic compounds (VOCs), thus significantly mediating partner attraction. A bacterial involvement in this mutualistic relationship could alter the manner in which these and similar relationships are viewed and evaluated. Future studies into mutualism stability and function among macroscopic partners will likely need to transition from a two-partner perspective to a multiple-partner perspective, and consider the microbial component, with the potential for one or more taxa making significant contributions to the relationship.

Keywords: ant, aphid, mutualism, bacteria, VOC, honeydew

Citation : *Fischer CY, Lognay GC, Detrain C, Heil M, Grigorescu A, Sabri A, Thonart P, Haubruge E and Verheggen F (2015) Bacteria may enhance species association in an ant–aphid mutualistic relationship. Chemoecology 25: 223–232. doi: 10.1007/s00049-015-0188-3*

Introduction

Food-related mutualisms with ants that are based on sugar-rich production by one partner are numerous and diverse, ranging from within-insect cooperation, like many honeydew-producing insects, to inter-kingdom relations, like extrafloral nectaries produced by several plant species (Beattie 1985; Stadler and Dixon 2005). Aphid-ant interactions have long been considered the paradigm of mutualism and the subject of considerable historical and contemporary research. Small and defenseless, aphids are easy prey for numerous predators and parasitoids. However, some aphid species are frequently found in association with ants that tend and protect the aphids in exchange for honeydew, which is a reliable and abundant carbohydrate source (Dixon 1985; Bristow 1991; Stadler and Dixon 2005). It has been estimated that myrmecophily*, i.e.* ants tending, is mandatory for one third of aphid species, facultative for another third, and never observed for the final third (Stadler 1997).

In Europe, three ant genera appear to be primarily involved in these mutualistic interactions: *Lasius, Myrmica* and *Formica* (Stadler and Dixon 1999; Guénard *et al.* 2007). Among mutualistic ant species, the black garden ant, *Lasius niger* L. (Hymenoptera, Formicidae), is well known to tend several aphid species, including the black bean aphid, *Aphis fabae* Scopoli (Homoptera, Aphididae) (El-Ziady and Kennedy 1956). These two species were selected as model taxa for this research as they are widespread in their natural environment and are frequently used as model species for the study of mutualistic relationships.

Honeydew, excreted in large amounts when aphids feed on the plant phloem sap, is a complex mixture of sugars, organic acids, amino acids and some lipids (Mittler 1958; Hussain *et al.* 1974; Leroy *et al.* 2011b). Its composition varies according to several factors, such as species, physiological state of the host plant, season and aphid endosymbionts (Fischer and Shingleton 2001; Fischer *et al.* 2002; Fischer *et al.* 2005; Woodring *et al.* 2007; Leroy *et al.* 2011b). Phloem sap composition is reflected in honeydew sugars and amino acids, the latter also being produced by endosymbiotic bacteria to palliate the very low and unbalanced concentrations of these compounds in the phloem sap (Douglas 1998; Febvay *et al.* 1999; Leroy *et al.* 2011b).

Excreted and deposited on the host plant or falling onto close surroundings, honeydew constitutes an excellent growth medium for diverse microorganisms, including potential aphid pathogens. Volatile compounds found in honeydew, mainly bacterial in origin, are known to attract aphid enemies such as the hoverfly *Episyrphus balteatus* (De Geer)(Leroy *et al.* 2011a).

Despite the potential negative biotic threats generated by honeydew, its benefits to the aphids include firming the mutualistic relationship with ant species that utilize the stable sugar source (Bristow 1991), in exchange for diverse services. Ant attendance is known to improve aphid fitness by increasing their reproductive rate and fecundity, extending life span and providing better protection against pathogens and enemies (El-Ziady and Kennedy 1956; Way 1963; Fischer *et al.* 2001; Stadler *et al.* 2001; Guénard *et al.* 2007). Therefore, while honeydew volatiles may attract aphid enemies, the compounds may also attract the species that benefit the aphids. Little is known about the specific role or magnitude of the beneficial contribution of the volatile chemical component to the mutualistic relationship. This paper describes the results of a study designed to investigate the role of these compounds in the attraction of potential ant partners and the origins of those relationships.

Material and methods

Plants and insects

In a climate-controlled room (16 h light-8h dark photoperiod; 20 ± 2°C), black bean aphids, *A. fabae* (Scopoli), were reared for several generations on broad beans, *Vicia faba* L., cultivated on a 1/1 mix of perlite and vermiculite substrate. Plants used in experiments measured around 15 cm high. All substrates used in behavioral assays were previously sterilized. *Lasius niger* (Linne) colonies were collected in the region surrounding Gembloux (Belgium), and kept under the same climatic conditions, but in separate chambers. Nests were placed in plastic containers coated with polytetrafluoroethylene (Fluon®,Whitford, U.K.) to prevent escape. Test tubes covered with a red transparent foil were used as laboratory rearing nests; a water and aqueous brown sugar solution (342 g/L) was provided in excess, and dead insects (fruit flies and mealworms) were provided weekly as an additional food source. All nests used in the bioassays were comprised of a queen, brood, and a minimum of 500 foragers.

Identification of the honeydew volatile compounds

The composition of the volatile organic compounds (VOCs) was determined using 250 µL glass inserts and solid-phase microextraction (SPME, 10 mm fiber with a 50/30 µm carboxen-divinylbenzene-polydimethylsiloxane coating – Supelco). The material analyzed was *A. fabae* honeydew (10 µL), dripping from aphid-infested *V. faba* plants, that was immediately collected on sterilized plastic foil under sterile conditions using 2 µL microcapillaries. Empty inserts were used for controls. Four replicates were conducted.

For each analysis, the same fibers were conditioned at 250°C for 1 h in a split-splitless injector before sampling. Volatile collections were performed at 25°C for 24 h. Desorption and GC-MS analyses were conducted on a Thermo Trace GC coupled with a Trace MS (Thermo Electron Corporation, Interscience, Louvain-la-Neuve, Belgium) equipped with an Optima 5 Accent (Macherey-Nagel, Düren, Germany) capillary column (30 m x 0.25 mm I.D.; 0.25 µm film thickness) under the following conditions: splitless injector at 230°C; vector gas was helium, at a 1 mL/min flow rate; oven temperature program: 40°C held for 2 min, raised at 5°C/min to 150°C, then at 10°C/min to 210°C, and finally at 120°C/min to 280°C held for 1 min; transfer line was at 250°C. Mass spectra were acquired at 70eV on a mass range from m/z 35 to 450 amu and analyzed using the NIST05 and Wiley8 libraries. Identifications were confirmed either by comparison with retention times of synthetic standards (Sigma-Aldrich, Steinheim, Germany) or by determination of retention indices. The relative proportions of each of the identified components are expressed in percent of total sample-related peak area.

Choice tests with two-way olfactometer

The degree of attractive effect on ants was assessed for different samples by using a two-way olfactometer, consisting of a Y-shaped glass tube (diameter: 1.5 cm, entrance length: 20 cm; length of each arm: 30 cm). Samples and controls were placed in 4 L glass jars. Filtered air was forced into the jars at 200 mL/min and delivered to the olfactometer's branches via Teflon® tubing.

Ants were starved for three days prior to an assay. The olfactometer's entrance was placed in the ant's rearing box, allowing the insects to enter the system. Only one ant worker at a time was allowed in the olfactometer; the entrance was closed to prevent additional ants from entering after the first ant went in. For each tested ant the following parameters were recorded: (1) the first branch it entered (initial orientation), (2) the choice, *i.e.* the branch selected to reach the end of the olfactometer arm and (3) the average linear speed while passing through the branch. The test ended when the ant reached a point located 25 cm from where the two arms branched ("choice point"). The linear speed was calculated by measuring the time spent to pass through an olfactometer branch, and was expressed in cm/s. The attractiveness of a sample was expressed by the relative number of ants (%) choosing the particular sample side as their final choice.

All assays were conducted at 20°C in a dark-walled chamber presenting no visual cues that may influence ant choices. To prevent ants from laying trails, they were never allowed to reach the actual samples. Moreover, in order to palliate any potential bias induced by the environment or by any marking of the substrate by exploring ants, sample and control sides were switched every five ants. The olfactometer was completely cleaned every 20 ants.

Several sample-control couples were tested following this protocol (Table 1), and changed every 20 ants. The first group of samples included naturally occurring elements. The goal was to assess to what extent each element that characterizes an aphid-infested plant contributes to its global attractiveness to the ants, specifically, the aphids themselves, the attacked plant and the honeydew. Since those elements cannot be completely dissociated, assays have been conducted by successive elimination of each element in order to assess their relative impact on ants' attraction. The first sample was an aphid infested plant (a pot holding nine *V. faba* infested by 50 *A. fabae* for three days); its attractiveness was compared to that of a healthy plant without aphids. The second sample was prepared similarly to the first one, but all aphids were completely removed before the assay. The last sample consisted of only honeydew collected for three days from a heavily infested plant onto wet substrate (perlite/vermiculite 1:1) to avoid desiccation. In this case, the control was a clean pot of substrate with the same humidity.

Table 1| Summary of samples and controls used in the behavioral assays

	Sample	Control	Number of tested ants
Naturally occuring elements	Aphid infested plant	Healthy plant	100
	Aphid infested plant, aphid removed	Healthy plant	100
	Honeydew, collected on wet substrate	Clean wet substrate (perlite/vermiculite 1:1)	120
Microbial cultures	Honeydew-inocculated 863 medium	Sterile 863 medium	60
	S. xylosus-inoculated 863 medium	Sterile 863 medium	60

The second group of samples included only bacterial cultures to assess the degree to which ants would be attracted to honeydew microflora. The first sample of this group was an 863 liquid culture medium, containing 20 g of glucose and 10 g of both yeast extract and casein peptone per liter of distilled water, inoculated with 20 µL of fresh honeydew collected as previously described and incubated for 2 d at 20°C. Sterile 863 medium was used as the control for this test. The second sample was the same medium inoculated solely with *Staphylococcus xylosus*, which is the only bacterial strain from *A. fabae* honeydew that was shown to have a significant attraction potential for the ants. This *S. xylosus* medium was incubated under the same conditions as the whole bacterial culture treatment, and the control was also sterile 863 culture medium. Sample volume for each test was 60 mL.

The significance of the ant preferences were assessed with binomial tests. Average linear speeds of ants in the two branches were compared, assay by assay, with t-tests. Global mean speeds observed for naturally occurring samples were compared by Kruskal-Wallis tests, which were also used to assess significance in the bacterial culture sample studies. If no differences were observed within a group, values within a group were pooled for between group comparisons using Kruskal-Wallis tests. Non-parametric tests were used when parametric assumptions were not observed. Differences were considered significant at $P \leq 0.05$. Statistical analyses were conducted using Minitab 15.1 (State College, Pennsylvania, USA).

Isolation of honeydew microbial content

Several bacteria which may influence the attractive effect of honeydew were isolated. Collected *Aphis fabae* honeydew (20 µL) was diluted and plated on 868 agar medium (containing, per liter of distilled water, 17 g of agar and 10 g each of glucose, yeast extract and casein peptone). Colonies were visible after 24 h of incubation at 25°C and the strains were isolated on the same medium based on morphological growth characteristics. The isolated strains were screened for their relative attractiveness.

In order to investigate the occurrence of additional fungal microorganisms potentially hidden by bacteria, the same process was repeated using chloramphenicol-treated plates. Chloramphenicol is a broad-band antibiotic specific against bacteria but not fungi, thus leaving only the latter as potentially influencing attractiveness.

Screening of microbial attractiveness

Once isolated, strains were cultured at 25°C in 863 liquid medium. After 48 h, attractiveness was tested by the same olfactometry protocol. These preliminary screenings were run with 20 ants per bacterium. Significance of the observed attractions was assessed with binomial tests using Minitab 15.1 software.

Identification of the attractive bacterium

For bacterial identification, genomic DNA was extracted from cells grown at 20°C for 48 h on 868 agar plates using a Wizard Genomic DNA purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

The 16S rRNA gene was PCR-amplified using the universal primers P0 (5'-GAA GAG TTT GAT CCT GGC TCA G-3') and P6 (5'-CTA CGG CTA CCT TGT TAC GA-3') (Ventura *et al.* 2001). The PCR reactions were completed using 1x ReadyMix Taq PCR Reagent Mix (Sigma-Aldrich, St. Louis, MO, USA), 0.5 µM of each primer and ~ 50 ng of genomic DNA as the template. The PCR program included a 5 min initial denaturation step at 95°C, followed by 26 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 2 min, and a final extension for 10 min at 72°C. The PCR product was purified using a GFX PCR DNA and Gel Band Kit (GE Healthcare, Buckinghamshire, UK), then sequenced using the Big Dye v3.1 Kit and an ABI 3730 DNA Analyzer (Applied Biosystems/Life Technology, Carlsbad, CA, USA) at the GIGA Center at the University of Liege. The same P0 and P6 primers were also used for sequencing. Electropherograms of both 16S gene strands were aligned, analyzed and edited with BioEdit (version 5.0.9; (Hall 1999)) in order to determine the correct 16S sequence.

Strain identification was conducted by comparing the 16S rRNA gene sequence determined here with all those in the GenBank database, using BLASTN (Altschul *et al.* 1997). The 16S sequence obtained here was deposited in the GenBank database under accession number KP668813.

In order to discriminate between the two identified *Staphylococcus* species, a diagnostic PCR was performed with primers that were specific for each of the two *Staphylococcus* species. Three PCR primer sets were used, one that was specific for *S. saprophyticcus*, 5'-TCA AAA AGT TTT CTA AAA AAT TTA C-3' and 5'-ACG GGC GTC CAC AAA ATC AAT AGG A-3' (Martineau *et al.* 2000), yielding a 210 bp fragment, and two sets that were specific for *S. xylosus*, XYL F (5'-AAC GCG CAA CGT GAT AAA ATT AAT G-3') and XYL R (5'-AAC GCG CAA CAG CAA TTA CG-3') (Morot-Bizot *et al.* 2003), yielding a 539 bp fragment, and xylBF (5’-CGT CTC AAG AAG TTG AAG ACA-3’) and xylBR (5’-CTC CAC CAC CAA TTG ATA CA-3’) (Blaiotta *et al.* 2003), yielding a 899 bp fragment. The PCR reactions were prepared as described above and the PCR program was the same as for amplification of the 16S rRNA gene, except that the elongation time was 1 min for the 899 bp fragment and 40 sec for the two smaller fragments. The PCR products were resolved by electrophoresis on 1% (w/v) agarose gels stained with 1 µg/mL ethidium bromide.

Identification of S. xylosus origin

To determine whether *S. xylosus* is excreted with honeydew or already present in the aphid environment, the whole bodies of 20 *A. fabae* adults were surface-sterilized by dipping into 70% ethanol for three minutes followed by washing in a sterile 9 g/L NaCl solution following the protocol proposed by Leroy *et al.* (Leroy *et al.* 2011a). Surface-sterilized aphids were crushed under sterile conditions in the same solution (using a previously described method (Fischer and Lognay 2012)), diluted and plated. Based on the morphology of the colonies, catalase activity and cell morphology, *S. xylosus* was isolated and its identification confirmed using API® Staph galleries (BioMérieux, Marcy l’Etoile, France).

Characterization of the S. xylosus VOC emission profile

In order to determine the contribution of *S. xylosus* in the emission of honeydew volatile compounds, SPME and GC-MS analyses were performed on bacterial cultures using a protocol proposed by Leroy *et al.* (Leroy *et al.* 2011a) and successfully applied to *Acyrthosiphon pisum* (Harris) bacteria VOC sampling (Table 2).

The bacterium was grown in 863 liquid culture medium at 20°C for 48 h. A 1 mL aliquot of the culture medium was placed in a 20 mL SPME vial. Control samples were 1 mL aliquots of sterile medium placed in similar vials. Volatile compounds were sampled by 10 mm DVB-CAR-PDMS SPME fibers (50/30 µm film thickness) (Supelco) for 3 h at 25°C. GC-MS analyses were conducted under the same conditions as previously described. Three replicates were analyzed.

Results

Aphid honeydew and ant attraction

Sixty-eight percent of the tested foragers were attracted by the complete infested plant system (host plant + aphids + honeydew; binomial test, n=100, p<0.001) (Fig 1); 64% of tested ants were attracted to a broad bean plant covered with honeydew (binomial test, n=100, p=0.007). Finally, 64% of the tested ants were attracted to honeydew alone (binomial test, n=120, p=0.002). The attraction toward honeydew alone was not statistically different from the complete system (χ^2 test, p = 0.391).

While no ant reversed course and tried to leave the olfactometer once they were inside, there were a few that changed their initial choice (Fig. 1). The proportion of ants that altered their choices appears to be higher for ants initially choosing the control side. During all those assays, ants' mean speed was monitored in both branches of the olfactometer (Fig. 1). No significant differences in speed were observed between sample and control branches within a test (t-test, all p>0.440), nor between the three tests described above (Kruskal-Wallis test, p=0.187).

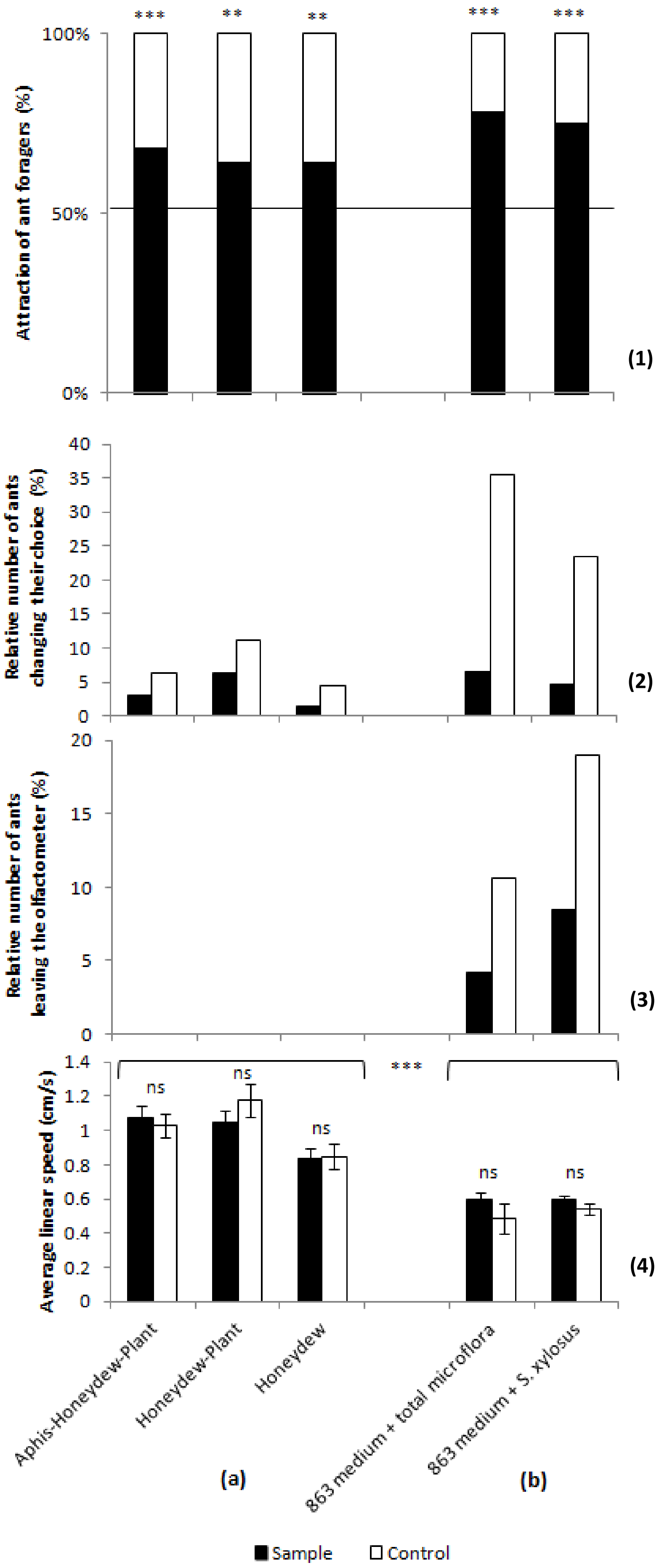


Fig. 1 Behavioral response of *L. niger* foragers to aphid related VOCs

Five preference tests of *L. niger* foragers in a two way-olfactometer presented with VOCs originating from (1) *A. fabae* infested plants (n=100), (2) infested plants with aphids just removed (n=100), (3) *A. fabae* honeydew (n=120), (4) 863 medium inoculated with all honeydew microflora (n=60), or (5) 863 medium inoculated with only *S. xylosus* (n=60). Controls for these five treatments were (treatments 1 and 2) healthy plants, (treatment 3) sterile wet culture substrate (perlite/vermiculite 1:1), and (treatments 4 and 5) sterile 863 medium. (1) Ants that choose either the Sample or Control branches as their final destination in the olfactometer (%). (2) and (3) Relative number of ants changing their initial choice (%) and leaving the olfactometer (%), respectively. (4) Linear speed (cm/s, X + SEM) of ants in each olfactometer branch. ** and *** indicate significant differences from the control at P<0.01 and P<0.001, respectively. ns = not significantly different (P>0.05).

Attractiveness of honeydew: the role of microorganisms

The biological effects of microbial VOCs (mVOCs) produced by honeydew bacteria were tested using the same olfactometer. Liquid 863 culture medium inoculated with freshly collected honeydew attracted a significantly higher number of ants, with 78% of the ant foragers following the sample branch (binomial test, n=60, p<0.001) (Fig. 1). Microbes were the only possible emitters of attractive VOCs since the control sample was sterile 863 culture medium inoculated with honeydew filtered through 0.2 µm membrane filters. It is highly unlikely therefore, that honeydew enzymes would have played a role in attracting the ants.

Once in the olfactometer, some ants left the system before reaching the end. The proportion of exiting ants appears higher for the control (11%) than for the sample (4%) branch. Moreover, as previously observed, the proportion of ants changing their choices appeared higher when the ants initially started down the control arm (36% *vs.* 7%). Compared to the earlier experiments, the average linear speed achieved by the ants decreased significantly (Kruskal-Wallis test, p<0.001), which is due to a more sinuousroute in the olfactometer with more brief stops. However, there remained no significant difference between control and sample sides (t-test, p=0.442).

Further behavioral assays performed with *S. xylosus* (as discussed in the next paragraph), and conducted using the same protocol as previously described, confirmed the attractiveness of this bacterium found in honeydew. Indeed, 75% of tested ants showed an attraction to the bacterium (n=60, binomial test, p<0.001).Tendencies observed with the honeydew-inoculated medium remained the same. However, the cases where ants reversed course still occurred more frequently when ants initially chose the control branch. Average linear speeds toward the bacterial samples were not statistically different (Kruskal-Wallis test, p=0.275), but were slower than the speeds exhibited by ants moving towards the complete infested plant system and its components (first three tests described above) (Kruskal-Wallis test, p<0.001). In addition to a slower (although not statistically slower) speed, ants were apparently more hesitant, with more brief stops. This speed remained equivalent for both branches of each assay (t-test, all p>0.360).

Identification and origin of attractive bacteria identified from honeydew

Six morphologically different bacteria were isolated from honeydew on 868 culture media. Yeasts and fungi were not present on this medium, either before or after adding the broadband antibacterial chloramphenicol.

Preliminary attraction potential screening showed a significant attractiveness for "bacterium 4" only (binomial test, $p<0.001$) (Fig. 2), with 90% of ants attracted. That bacterium, found at a concentration of $2x10^5$ CFU/mL of honeydew, was identified as *Staphylococcus* sp. (potentially *saprophyticcus* or *xylosus*) by 16S ribosomal RNA sequences based on 100% nucleotide sequence identity with these two species over a 100% sequence coverage. The diagnostic PCR amplification was negative with the *S. saprophyticcus*-specific primers and positive with the two sets of *S. xylosus*-specific primers, confirming that the isolated strain was *S. xylosus*.

The occurrence of the bacterium in surface-sterilized aphid bodies was demonstrated by an API Staph test (BioMérieux) after isolation of the strain.

Origin of the honeydew attractive VOCs

Twenty-two volatile chemicals, including ketones, esters, alcohols, aldehydes, and acids, were identified from freshly collected honeydew (Table 2). Most of them were also detected in *S. xylosus* cultures and were similar to the mVOCs produced by *S. sciuri,* an *A. pisum* bacterium (Leroy *et al.* 2011a) (Table 2).

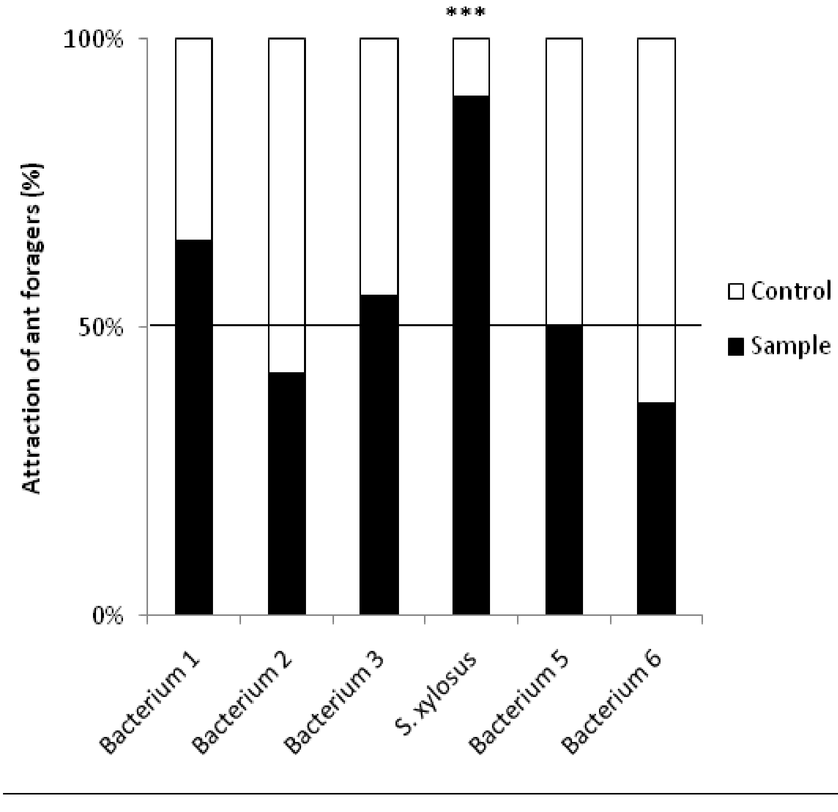


Fig. 2 Screening of A. fabae honeydew bacteria for their potential to emit mVOC's attractive to the ants

Samples are bacteria-inoculated 863 liquid culture medium; controls are sterile aliquots of the same medium. *** indicates a significant difference from the control at $P<0.001$ (binomial test).

Table 2| Volatile organic compounds (VOCs) found in aphid-secreted honeydew and bacterial-inoculated medium

Retention time (min)	VOC	*A.fabae* honeydew	*S. xylosus*-inoculated 863 liquid medium	*A. pisum* honeydew*	*S. sciuri*-inoculated 863 liquid medium*
1.65	Propanone	0.85 ± 0.14	0.98 ± 0.02	9.25 ± 2.99	15.58 ± 6.46
1.78	Methyl acetate	6.75 ± 3.83			
2.05	2,3-Butanedione	0.45 ± 0.17	0.27 ± 0.03	2.31 ± 1.26	3.14 ± 1.74
2.22	Ethyl acetate	19.95 ± 15.11			
2.39	2-Methylpropanol	0.67 ± 0.25			
2.61	3-Methylbutanal		1.54 ± 0.32	14.01 ± 3.24	
2.71	2-Methylbutanal		1.91 ± 0.02	12.92 ± 1.33	
2.97	Ethanoic acid	1.47 ± 0.82	46.36 ± 0.73		
3.50	1-Methylethyl acetate	0.10 ± 0.03			
3.57	3-Hydroxy-2-butanone	0.05 ± 0.05		0.78 ± 0.24	3.19 ± 1.34
3.70	3-Methyl-3-buten-1-ol	0.28 ± 0.13	0.05 ± 0.01	0.89 ± 0.39	
3.80	3-Methyl-1-butanol	5.40 ± 3.41	16.45 ± 1.03	12.32 ± 5.58	24.75 ± 12.75
3.87	2-Methyl-1-butanol	1.73 ± 0.35	4.43 ± 0.12		
4.52	2-Methylpropanoic acid		1.20 ± 0.04		
4.57	2-Methylpropyl acetate	0.38 ± 0.20			
5.51	Butanoic acid	0.05 ± 0.03	5.43 ± 0.17	6.24 ± 3.45	12.89 ± 4.67
7.06	3-Methylbutanoic acid	1.93 ± 1.13	7.88 ± 0.10	4.56 ± 0.45	9.53 ± 3.56
7.18	2-Methylbutanoic acid	0.99 ± 0.70	0.93 ± 0.00	6.73 ± 5.55	9.76 ± 2.87
7.28	3-Methyl-1-butyl acetate	1.03 ± 0.44	0.18 ± 0.02		
7.36	2-Methyl-1-butyl acetate	0.46 ± 0.15	< 0.01		
8.32	2,5-Dimethylpyrazine		1.41 ± 0.03	0.31 ± 0.16	3.10 ± 1.14
9.83	Benzaldehyde	0.14 ± 0.07	5.18 ± 0.19		
10.58	6-Methyl-5-heptene-2-one		4.00 ± 0.49		
12.04	2-ethyl-1-hexanol	0.17 ± 0.02			
12.42	Benzeneacetaldehyde	0.40 ± 0.13	0.63 ± 0.10		
14.18	Linalool		0.37 ± 0.02		
14.56	Benzeneethanol	53.12 ± 16.40	0.82 ± 0.15	1.73 ± 0.50	3.60 ± 1.43
17.81	Phenylethyl acetate	3.65 ± 1.89			
	2-Methyl-2-buten-1-ol			14.41 ± 1.39	
	3-Methyl-2-butenal			10.73 ± 2.71	14.46 ± 6.34
	Limonene			2.81 ± 0.17	

Relative proportions (%±s.e.m.; honeydew: n=4; cultures: n=3) of the volatile compounds collected by solid-phase microextraction and analyzed by gas chromatography-mass spectrometry.

* Data from Leroy *et al.* (2011a)

Discussion

Honeydew is the keystone factor leading to ant-aphid partnerships, strongly influencing the relationship through its composition and abundance (Way 1963; Kiss 1981; Völkl *et al.* 1999; Fischer *et al.* 2001). The data presented here represent the first evidence of a distant attraction of ants that is driven by aphid honeydew and mediated by the associated microflora through release of VOCs, in laboratory conditions. Even though mVOCs emitted from honeydew have been reported to attract aphid enemies (Leroy *et al.* 2011a), the potential extent of their role in ant-aphid mutualism was unexpected. Indeed, when considering the plant-aphid-honeydew system, neither the removal of plants nor the aphids themselves significantly influenced ant behavior. Honeydew VOCs alone have the same biological effects as the complete system (Fig. 1). However, previous studies have shown an effect from diverse concentrations of E-β-farnesene (EBF), an aphid pheromone, on ant social bebavior (Nault *et al.* 1976; Verheggen *et al.* 2012). In combination with our results, this suggests either that VOCs emitted by the aphid itself (EBF) present a relatively low attractiveness for the ants compared to honeydew or their effect is not additive with that of the honeydew; honeydew alone being able to elicit the entire response.

Honeydew-inoculated, as well as *S. xylosus*-inoculated, culture media remained attractive, confirming the high importance of honeydew microorganisms.

When comparing results from behavioral assays, two groups can be distinguished: one composed of naturally occurring samples (the aphid infested plant and its separate components), and one comprised of bacterial cultures (Fig. 1). While attractiveness remains constant throughout all assays, for the last group the number of ants leaving the initially-selected branch was higher and their average speed was lower. Those results suggested a reduction of the efficiency of ant attraction which is likely a result from the differences of VOC profiles observed between honeydew and bacterial cultures (Table 2), which probably originates from the differences between those two groups in terms of bacterial growth conditions.

When isolated from *A. fabae* honeydew microflora and from surface-sterilized aphids, *S. xylosus* emits a mVOC pattern qualitatively similar to that produced by genuine honeydew excreted by *A.fabae* colonies or to that emitted by cultures of *S. sciuri* (Table 2). The latter, isolated from the non-myrmecophilous pea aphid *Acyrtosiphon pisum*, and closely related to *S. xylosus*, is known to release mVOCs which guide hoverflies towards their aphid prey

(Leroy *et al.* 2011a). Of the 10 compounds described by these authors, nine have also been found in *S. xylosus* mVOCs, and 10 new compounds have been identified for the latter species (Table 2). Several of them have been found in diverse glands of various ant species, mostly Myrmicinae. The identified compounds include propanone (Attygalle *et al.* 1983) and 2-methyl and 3-methlybutanoic acid (Wood *et al.* 2002), which have been found in Dufour glands as well as in mandibular glands (Cammaerts *et al.* 1981; Wood *et al.* 2011). These glands have also been found to contain 3-methyl-1-butanol, butanoic acid (Wood *et al.* 2002) and phenylethanol (Wood *et al.* 2011), the latter having also been found in poison glands, along with 2, 5-dimethylpyrazine; phenylethanol is involved in recruitment ant trail laying (Attygalle and Morgan 1984; Liu and Liu 2002; Plowes *et al.* 2014). Benzaldehyde has been found in pygidial glands (Hölldobler *et al.* 2013). However, none of these compounds have previously been found in the genus *Lasius*, nor identified as semiochemicals for this genus. Aphid gut microflora is partially acquired during probing by the insect on the host leaf surface or feeding in vascular tissues. Bacteria are able to pass through the stylets' food canal, colonize the luminal surface of intestinal epithelia, and be partially excreted in honeydew (Grenier *et al.* 1994; Harada and Ishikawa 1997; Davidson *et al.* 2000; Leroy *et al.* 2011a). Since honeydew had been collected in sterile conditions immediately after its excretion, *S. xylosus*, found at a concentration of $2x10^5$ CFU/mL of fresh honeydew, can be considered a host-associated bacterium excreted by the aphid rather than a bacterium colonizing honeydew after its excretion. *Staphylococcus xylosus* has been found in surface-sterilized aphids following a protocol proposed by Leroy *et al.* 2011a, thus confirming the host origin of this bacterium. Moreover, *S. xylosus* is a quite ubiquitous species, found in various natural environments like soil, beach sand, water, grass and the skin of cattle and birds (Kloos 1980). Aphids are likely hosts of this bacterium, which is probably acquired during probing (Haynes *et al.* 2003). Aphid physical and biochemical mechanisms, as well as the composition of honeydew, could be effective filters, allowing only a few bacterial taxa to develop and colonize the gut; similar to what occurs for yeasts in flower nectar (Herrera *et al.* 2010). This kind of partner selection is based on the microbes' abilities to survive in this rather hostile environment (high osmotic pressure, low oxygen, antimicrobial compounds produced by the host) and on the affinity of the microorganism for its host (González-Teuber and Heil 2010; Herrera *et al.* 2010; Álvarez-Pérez *et al.* 2012; Kirzinger and Stavrinides 2012).

The volatile compounds identified from plain honeydew or honeydew-isolated bacteria probably originate from degradation processes and/or modifications of sugars and amino acids of the substrate. It has been shown that direct modifications, especially those made by *Staphylococcus* sp. on several amino acids such as leucine, isoleucine and valine, can produce a whole set of C4 and C5 components with different chemical functions, from aldehydes to their corresponding alcohols, acids and esters (Thibout *et al.* 1993; Schulz and Dickschat 2007). This would explain the origin of most identified non-cyclic compounds reported in Table 2. For example, this pathway is probably responsible for the production of 2-methylbutanal and 3-methylbutanal, as well as their corresponding alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) and acids (2-methylbutanoic acid and 3-methylbutanoic acid). Also synthesized by *S. sciuri* isolated from the aphid *A. pisum*, these last two compounds are known to attract and induce egg-laying by the aphid predator hoverfly *Episyrphus balteatus* (Leroy *et al.* 2011a). They may also be potentially involved in ant attraction, as other species use them to signal the presence of aphids. In situations of amino acid scarcity, which is the case for honeydew, *S. xylosus* is also able to form acids from glucose *via* another biosynthetic pathway involving pyruvate (Beck *et al.* 2004). Aromatic compounds, like benzeneethanol, are produced by degradation of aromatic amino acids. Along with 3-hydroxy-2-butanone, 2,3-butanedione and 3-methyl-3-buten-1-ol, these are typical fermentation-associated substances (Thibout *et al.* 1993; Schulz and Dickschat 2007; Leroy *et al.* 2011a). Therefore, despite widespread distribution in the environment, *S. xylosus* remains a specific indicator of honeydew presence through its mVOC emissions modulated by the growth substrate.

The ability to associate these mVOC patterns and quantities with potential aphid partners is likely to aid ant scouts by providing information about the aphid species, their location and productivity through the amount of mVOCs and the modifications of mVOC patterns of ageing honeydew. Ant scouts may then exploit this information to refine their search patterns and selection of new aphid partners.

Many widespread cases of mutualism are based on the ability of one partner to produce sugar-rich secretions, exploited by the other. For example, nectaries-bearing plants attract ants and thus enhance their protection against other insects (González-Teuber and Heil 2010) and flowers that are rich in nectar attract various pollinators (e.g., insects, birds and small mammals). Sugary secretions, like nectars, also constitute a suitable growth medium for diverse microorganisms. Indeed, yeasts and bacteria have been repeatedly isolated from the nectar of flowers pollinated by various organisms including insects, birds and small mammals (Wiens *et al.* 2008; Herrera *et al.* 2009; Álvarez-Pérez *et al.* 2012; Vannette *et al.* 2013). Microbial presence alters the composition of the nectar and can modify its VOC production, thus influencing pollination. Effects on the plant (e.g., sugar modification, ethanol production) are either positive or negative for the relationship, depending on the extent of these modifications, the microorganisms involved and the pollinators considered (Herrera *et al.* 2008; Wiens *et al.* 2008; Herrera *et al.* 2009; Vannette *et al.* 2013). While yeasts do not seem to be detrimental for plant-pollinator mutualisms at low infestation levels, bacteria are able to weaken these relationships (Vannette *et al.* 2013).

The data presented here indicated that, apart from serving as a source of nutrients, certain microbes in honeydew are attractive to ants. This first evidence of the influence of microbes in ant-aphid mutualism contributes to an altered perspective of the importance of bacterial volatiles in animal interactions. Microorganisms can impact mutualistic relationships through both direct substrate modifications as well as through emissions of volatile compounds. These results emphasize the importance of investigating the presence and potential effects of microbes in insect symbioses.

Acknowledgments

The authors wish to thank Dr. M. Vanderplanck, MSc H. Cawoy, Mr O. Piraux and Mr P. Hacourt for their support during this project. Christophe Fischer is financially supported by a PhD grant from the *Fonds pour la formation à la Recherche dans l'Industrie et l'Agriculture* (FRIA). This project is also financially supported by a *Fonds de la Recherche Fondamentale Collective* (F.R.F.C. – F.N.R.S.) research project (2.4600.09).

References

Altschul SF, Madden TL, Schäffer AA, et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. doi: 10.1093/nar/25.17.3389

Álvarez-Pérez S, Herrera CM, de Vega C (2012) Zooming-in on floral nectar: A first exploration of nectar-associated bacteria in wild plant communities. FEMS Microbiol Ecol 80:591–602. doi: 10.1111/j.1574-6941.2012.01329.x 10.1111/j.1365-294X.2011.05402.x

Attygalle AB, Evershed RP, Morgan ED, Cammaerts MC (1983) Dufour gland secretions of workers of the ants Myrmica sulcinodis and Myrmica lobicornis, and comparison with six other species of Myrmica. Insect Biochem 13:507–512.

Attygalle AB, Morgan ED (1984) Identification of trail pheromone of the ant Tetramorium caespitum L. (Hymenoptera: Myrmicinae). J Chem Ecol 10:1453–1468.

Beattie AJ (1985) The Evolutionary Ecology of Ant–Plant Mutualisms. publisherNameCambridge University Press

Beck HC, Hansen AM, Lauritsen FR (2004) Catabolism of leucine to branched-chain fatty acids in Staphylococcus xylosus. J Appl Microbiol 96:1185–1193. doi: 10.1111/j.1365-2672.2004.02253.x

Blaiotta G, Pennacchia C, Parente E, Villani F (2003) Design and Evaluation of Specific PCR Primers for Rapid and Reliable Identification of Staphylococcus xylosus Strains Isolated from Dry Fermented Sausages. Syst Appl Microbiol 26:601–610. doi: http://dx.doi.org/10.1078/072320203770865918

Bristow CM (1991) Why are so few aphids ant-tended? In: Cutler DF, Huxley C (eds) Ant/Plant Interactions. Oxford University Press, pp 104–119

Cammaerts MC, Evershed RP, Morgan ED (1981) Comparative study of the mandibular gland secretion of four species of Myrmica ants. J Insect Physiol 27:225–231.

Davidson EW, Rosell RC, Hendrix DL (2000) Culturable bacteria associated with the whitefly, Bemisia Argentifolii (Homoptera: Aleyrodidae). Florida Entomol 83:159–171.

Dixon AFG (1985) Aphid ecology. Blackie ; Distributed in the U.S.A. by Chapman and Hall, Glasgow

Douglas AE (1998) Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria Buchnera. Annu Rev Entomol 43:17–37. doi: 10.1146/annurev.ento.43.1.17

El-Ziady S, Kennedy JS (1956) Beneficial effects of the common garden ant, Lasius niger L., on the black bean aphid, Aphis fabae Scopoli. Proc R Ent Soc Lond 31:5.

Febvay G, Rahbe Y, Rynkiewicz M, et al (1999) Fate of dietary sucrose and neosynthesis of amino acids in the pea aphid, acyrthosiphon pisum, reared on different diets. J Exp Biol 202 (Pt 19:2639–2652.

Fischer CY, Lognay GC (2012) Simple and Automatic Closed Grinding and Extraction System. J Chem Educ 89:1611–1612. doi: 10.1021/ed2007907

Fischer MK, Hoffmann KH, Völkl W (2001) Competition for mutualists in an ant-homopteran interaction mediated by hierarchies of ant attendance. Oikos 92:531–541.

Fischer MK, Shingleton AW (2001) Host plant and ants influence the honeydew sugar composition of aphids. Funct Ecol 15:544–550.

Fischer MK, Volkl W, Hoffmann KH (2005) Honeydew production and honeydew sugar composition of polyphagous black bean aphid, Aphis fabae (Hemiptera: Aphididae) on various host plants and implications for ant-attendance. Eur J Entomol 102:155–160.

Fischer MK, Volkl W, Schopf R, Hoffmann KH (2002) Age-specific patterns in honeydew production and honeydew composition in the aphid Metopeurum fuscoviride: implications for ant-attendance. J Insect Physiol 48:319–326.

González-Teuber M, Heil M (2010) Pseudomyrmex ants and acacia host plants join efforts to protect their mutualism from microbial threats. Plant Signal Behav 5:890–892.

Grenier AM, Nardon C, Rahbé Y (1994) Observations on the micro-organisms occurring in the gut of the pea aphid Acyrthosiphon pisum. Entomol Exp Appl 70:91–96. doi: 10.1111/j.1570-7458.1994.tb01762.x

Guénard B, Lucas É, Francoeur A (2007) Mutualisme fourmis pucerons et guilde aphidiphage associée : le cas de la prédation furtive. Université du Québec à Montréal

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98.

Harada H, Ishikawa H (1997) Experimental pathogenicity of Erwinia aphidicola to pea aphid, Acyrthosiphon pisum. J Gen Appl Microbiol 43:363–367.

Haynes S, Darby AC, Daniell TJ, et al (2003) Diversity of Bacteria Associated with Natural Aphid Populations. Appl Environ Microbiol 69:7216–7223. doi: 10.1128/aem.69.12.7216-7223.2003

Herrera CM, Canto A, Pozo MI, Bazaga P (2010) Inhospitable sweetness: Nectar filtering of pollinator-borne inocula leads to impoverished, phylogenetically clustered yeast communities. Proc R Soc B Biol Sci 277:747–754. doi: 10.1098/rspb.2009.1485 10.1034/j.1600-0706.2000.910301.x

Herrera CM, de Vega C, Canto A, Pozo MI (2009) Yeasts in floral nectar: a quantitative survey. Ann Bot 103:1415–1423. doi: 10.1093/aob/mcp026

Herrera CM, García IM, Pérez R (2008) Invisible floral larcenies: Microbial communities degrade floral nectar of bumble bee-pollinated plants. Ecology 89:2369–2376. doi: 10.1890/08-0241.1

Hölldobler B, Plowes NJR, Johnson RA, et al (2013) Pygidial gland chemistry and potential alarm-recruitment function in column foraging, but not solitary, Nearctic Messor harvesting ants (Hymenoptera: Formicidae: Myrmicinae). J Insect Physiol 59:863–869.

Hussain A, Forrest JM, Dixon AF (1974) Sugar, organic acid, phenolic acid and plant growth regulator content of extracts of honeydew of the aphid Myzus persicae and of its host plant, Raphanus sativus. Ann Appl Biol 78:65–73. doi: 10.1111/j.1744-7348.1974.tb01486.x

Kirzinger MWB, Stavrinides J (2012) Host specificity determinants as a genetic continuum. Trends Microbiol 20:88–93. doi: http://dx.doi.org/10.1016/j.tim.2011.11.006

Kiss A (1981) Melezitose, aphids and ants. Oikos 37:382.

Kloos WE (1980) Natural populations of the genus Staphylococcus. Annu Rev Microbiol 34:559–592.

Leroy PD, Sabri A, Heuskin S, et al (2011a) Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. Nat Commun 2:7. doi: Artn 348 Doi 10.1038/Ncomms1347

Leroy PD, Wathelet B, Sabri A, et al (2011b) Aphid-host plant interactions: does aphid honeydew exactly reflect the host plant amino acid composition? Arthropod Plant Interact 5:193–199. doi: DOI 10.1007/s11829-011-9128-5

Liu Y, Liu Y (2002) Identification of recruitment pheromones in the harvester ant genus Pogonomyrmex. Fenxi Huaxue 30:298–300.

Martineau F, Picard FJ, Ménard C, et al (2000) Development of a rapid PCR assay specific for Staphylococcus saprophyticus and application to direct detection from urine samples. J Clin Microbiol 38:3280–3284.

Mittler TE (1958) The feeding and nutrition of large willow aphid (Tuberolachnus salignus). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. J Exp Biol 35:74–84.

Morot-Bizot S, Talon R, Leroy-Setrin S (2003) Development of specific PCR primers for a rapid and accurate identification of Staphylococcus xylosus, a species used in food fermentation. J Microbiol Methods 55:279–286. doi: http://dx.doi.org/10.1016/S0167-7012(03)00159-3

Nault LR, Montgomery ME, Bowers WS (1976) Ant-aphid association: role of aphid alarm pheromone. Sci USA 192:1349–1351.

Plowes NJR, Colella T, Johnson RA, Hölldobler B (2014) Chemical communication during foraging in the harvesting ants Messor pergandei and Messor andrei. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol 200:129–137.

Schulz S, Dickschat JS (2007) Bacterial volatiles: The smell of small organisms. Nat Prod Rep 24:814–842. doi: 10.1039/b507392h

Stadler B (1997) The relative importance of host plants, natural enemies and ants in the evolution of life-history characters in aphids. In: Dettner K, Bauer G, Völkl W (eds) Vertical food web interactions : evolutionary patterns and driving forces. Springer - Verlag, pp 241–256

Stadler B, Dixon AFG (2005) Ecology and evolution of aphid-ant interactions. Annu Rev Ecol Evol Syst 36:345–372.

Stadler B, Dixon AFG (1999) Ant attendance in aphids: why different degrees of myrmecophily? Ecol Entomol 24:363–369.

Stadler B, Fiedler K, Kawecki TJ, Weisser WW (2001) Costs and benefits for phytophagous myrmecophiles: When ants are not always available. Oikos 92:467–478.

Thibout E, Guillot JF, Auger J (1993) Microorganisms are involved in the production of volatile kairomones affecting the host seeking behaviour of Diadromus pulchellus, a parasitoid of Acrolepiopsis assectella. Physiol Entomol 18:176–182.

Vannette RL, Gauthier MPL, Fukami T (2013) Nectar bacteria, but not yeast, weaken a plant - Pollinator mutualism. Proc R Soc B Biol Sci. doi: 10.1098/rspb.2012.2601

Ventura M, Elli M, Reniero R, Zink R (2001) Molecular microbial analysis of Bifidobacterium isolates from different environments by the species-specific amplified ribosomal DNA restriction analysis (ARDRA). FEMS Microbiol Ecol 36:113–121. doi: http://dx.doi.org/10.1016/S0168-6496(01)00123-4

Verheggen FJ, Diez L, Sablon L, et al (2012) Aphid Alarm Pheromone as a Cue for Ants to Locate Aphid Partners. PLoS One 7:e41841. doi: 10.1371/journal.pone.0041841

Völkl W, Woodring J, Fischer M, et al (1999) Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. Oecologia 118:483–491.

Way MJ (1963) Mutualism between ants and honeydew-producing Homoptera. Annu Rev Entomol 8:307–344.

Wiens F, Zitzmann A, Lachance MA, et al (2008) Chronic intake of fermented floral nectar by wild treeshrews. Proc Natl Acad Sci U S A 105:10426–10431. doi: 10.1073/pnas.0801628105

Wood WF, Hoang TT, McGlynn TP (2011) Volatile components from the mandibular glands of the turtle ants, Cephalotes alfaroi and Cephalotes cristatus. Biochem Syst Ecol 39:135–138.

Wood WF, Palmer TM, Stanton ML (2002) A comparison of volatiles in mandibular glands from three Crematogaster ant symbionts of the whistling thorn acacia. Biochem Syst Ecol 30:217–222.

Woodring J, Wiedemann R, Volkl W, Hoffmann KH (2007) Oligosaccharide synthesis regulates gut osmolality in the ant-attended aphid Metopeurum fuscoviride but not in the unattended aphid Macrosiphoniella tanacetaria. J Appl Entomol 131:1–7. doi: 10.1111/j.1439-0418.2006.01091.x

BACTERIA MAY CONTRIBUTE TO DISTANT SPECIES RECOGNITION IN ANT-APHID MUTUALISTIC RELATIONSHIPS

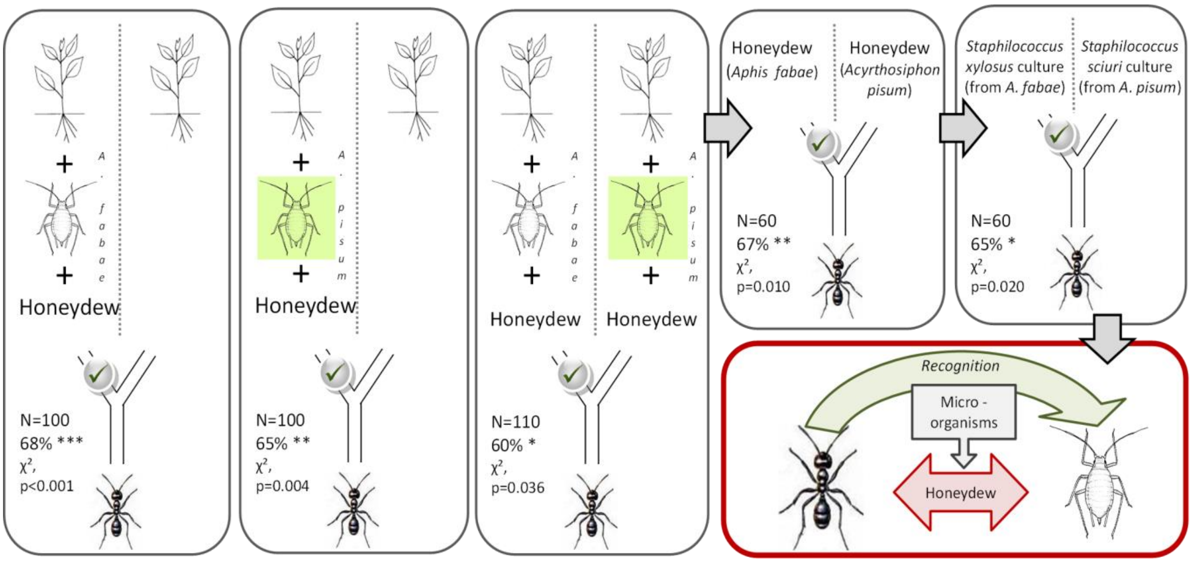
Christophe Y. Fischer[1*], Claire Detrain[2], Philippe Thonart[3], Eric Haubruge[4], Frédéric Francis[4], François J. Verheggen[4] and Georges C. Lognay[1]

[1] Laboratory of Analytical Chemistry, University of Liege, Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium.

[2] Unit of Social Ecology, Université Libre de Bruxelles, CP231,50 avenue F. Roosevelt, B-1050 Brussels, Belgium.

[3] Walloon Center of Industrial Biology, University of Liege, B40, 4000 Sart-Tilman, Belgium.

[4] Department of Functional and Evolutionary Entomology, University of Liege, Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium.



Graphical abstract - Distant mutualistic partner recognition based on VOC emissions of a microbial third party

Abstract: Mutualistic interactions between ant and aphid species have been the subject of considerable historical and contemporary investigations, the primary benefits being cleaning and protection for the aphids and carbohydrate-rich honeydew for the ants. Questions remained, however, as to the volatile semiochemical factor influencing this relationship. A recent study highlighted the role of bacterial honeydew volatile compounds in ant attraction. Here, ant's ability to distantly discriminate two aphid species was investigated based on bacterial honeydew semiochemical emissions using a two-way olfactometer. Both the mutualistic aphid *Aphis fabae* L. and the non-myrmecophilous aphid *Acyrthosiphon pisum* Harris were found to be attractive for the ant *Lasius niger* L. The level of attraction was similar in both assays (control versus one of the aphid species). However, when given a choice between these two aphid species, ants showed a significant preference for *Aphis fabae*. Honeydew volatiles, mostly from bacterial origins, are known to be a key element in ant attraction. Using the same olfactometry protocol, the relative attractiveness of volatiles emitted by honeydews collected from each aphid species and by bacteria isolated from each honeydew was investigated. Again, ants significantly preferred volatiles released by *Aphis fabae* honeydew and bacteria. This information suggests that microbial honeydew volatiles enable ants to distantly discriminate aphid species. These results strengthen the interest of studying the occurrence and potential impact of microorganisms in insect symbioses.

Keywords: ant, aphid, bacteria, honeydew, mutualism, recognition, VOC

Citation : *Fischer CY, Detrain C, Thonart P, Haubruge E, Francis F, Verheggen FJ and Lognay GC (2016) Bacteria may contribute to distant species recognition in ant-aphid mutualistic relationships. Insect Science, doi:10.1111/1744-7917.12302*

Introduction

Ant-aphid interactions, as a major model of mutualistic relationships, have been the subject of considerable historical and contemporary investigations. Small and defenseless aphids are easy prey for numerous predators and parasitoids. However, some aphid species are frequently found in association with ants that tend and protect the aphids in exchange of honeydew, which is a reliable and abundant carbohydrate source. Other aphid species do not develop such partnerships with ants and are rather considered as preys (Dixon 1985; Bristow 1991; Stadler and Dixon 2005).

In Europe, it has been estimated that myrmecophily*, i.e.* ants tending, is observed for two thirds of aphid species (Stadler 1997). Three ant genera appear to be primarily involved in these mutualistic interactions: *Lasius, Myrmica* and *Formica* (Stadler and Dixon 1999; Guénard *et al.* 2007), among which the black garden ant, *Lasius niger* L. (Hymenoptera, Formicidae), is well known to tend several aphid species, including the black bean aphid, *Aphis fabae* Scopoli (Homoptera, Aphididae) (El-Ziady and Kennedy 1956). These two species are frequently used in studies on mutualistic interactions. Besides, the pea aphid, *Acyrthosiphon pisum* Harris can be reared on the same host plant, *Vicia faba* L., as the bean aphid but is never observed in association with ants. For these reasons, these three insect species, widespread in their natural environment, were selected as model taxa for this research (Wilson 1955; Holman 2009).

While aphids do not seem to actively search for ant partners, ants are known to search for their aphid partners and are able to use different volatile cues to orientate this search, namely volatile organic compounds emitted by honeydew bacteria (mVOCs) and E-β-farnesene (EBF), an aphid pheromone involved in alarm and social behaviors (Verheggen et al. 2012; Fischer et al. 2015b; Fischer et al. 2015a). This last molecule is a major component of the alarm pheromone of numerous aphid species, and is thus not likely to constitute alone a suitable species recognition cue (Francis *et al.* 2005). In contrast, honeydew VOCs emission profiles vary between aphid species, depending on various factors including the aphid species and its gut microflora, and could thus contribute to distant aphid discrimination. This paper describes a study designed to investigate the role of honeydew mVOCs in distant aphid discrimination by ant partners.

Material and methods

Plants and insects

In climate-controlled rooms (16 h light-8h dark photoperiod; 20 ± 2°C), *Aphis fabae* and *Acyrthosiphon pisum*, were reared for several generations on *Vicia faba* plants cultivated on a 1/1 mix of perlite and vermiculite substrate. Plants used in experiments were about 15 cm high. All substrates used in biological tests were previously sterilized. *Lasius niger* colonies were collected in Gembloux (Belgium), and kept in separate chambers under the same conditions. To prevent escape, the nests (test tubes covered with a red transparent foil) were placed in plastic containers coated with polytetrafluoroethylene (Fluon®,Whitford, U.K.). Water and aqueous brown sugar solution (342 g/L) were provided *ad libitum*. Dead fruit flies and mealworms were provided weekly as a complementary food source. All nests were composed by a queen, brood, and a minimum of 500 foragers.

Biological tests and chemical investigations.

Choice tests with two-way olfactometer as well as characterization of honeydew's and bacterial VOCs emission profiles by SPME-GC-MS were undertaken according to Fischer *et al.* (2015a).

Samples and controls used in the behavioral assays are summarized in table 1. For each modality, samples and controls presented to ants were used to test 20 ants and then renewed. The first samples were aphid-infested plants (substrate with nine V. faba infested for three days either by 50 myrmecophilous *Aphis fabae* or by 50 non-myrmecophilous *Acyrthosiphon pisum* respectively); their attractiveness were compared to that of a healthy plants without aphids.

The following test assessed the relative attractiveness of the two first samples, plants infested by one of these two species being presented at each side of the olfactometer.

The global attractiveness of an aphid-infested plant relies mostly on volatile organic compounds (VOCs) released by the honeydew accumulating around aphid colonies, and more specifically by aphid-associated bacteria present in honeydew (Fischer *et al.* 2015a). The relative attractiveness of honeydews was thus assessed for the two tested aphid species. Sample consisted of *Aphis fabae* honeydew that was collected for three days from a heavily infested plant onto wet substrate (perlite/vermiculite 1:1) to avoid desiccation, while control was *Acyrthosiphon pisum* honeydew (collected the same way).

Table 1| Summary of samples and controls used in the behavioral assays

Sample	Control	Number of tested ants
Aphis fabae infested plant	Healthy plant	100
Acyrthosiphon pisum infested plant	Healthy plant	100
Aphis fabae infested plant	*Acyrthosiphon pisum* infested plant	100
Aphis fabae honeydew, collected on wet substrate	*Acyrthosiphon pisum* honeydew, collected on wet substrate	60
Staphylococcus xylosus-inoculated 863 medium	*Staphylococcus sciuri*-inoculated 863 medium	60

The relative attractiveness of two taxonomically close honeydew bacteria known to be involved in aphid interactions with other insect species was also tested. The first one, *Staphylococcus xylosus*, is found in *Aphis fabae* gut and honeydew and is known to produce mVOCs attractive for *L. niger* (Fischer *et al.* 2015a); the second one, *Staphylococcus sciuri*, is found in *Acyrthosiphon pisum* gut and honeydew and is known to attract *Episyrphus balteatus* (De Geer), an aphid enemy (Leroy et al. 2011a). Both these bacteria were found only in one of the two studied aphid species. Sample and control consisted in 60mL of 868 culture medium (20 g of glucose and 10 g of both yeast extract and casein peptone per liter of distilled water) inoculated with *S. xylosus* and *S. sciuri* respectively and incubated for 2 days at 20°C.

Binomial tests were carried out to assess the significance of the ant preferences. The significances of differences of attractiveness observed between tests were assessed by χ^2 test. Average linear speeds of ants in the two branches were compared, assay by assay, with t-tests. Differences were considered significant at $P < 0.05$. Statistical analyses were performed using Minitab 15.1 (State College, Pennsylvania, USA).

Results

VOCs and ant attraction

Plants infested by myrmecophilous and non-myrmecophilous aphid species significantly attracted ants when tested against healthy plants; respectively 68 % and 65 % of the tested foragers were attracted towards *Aphis fabae* and *Acyrthosiphon pisum* infested plants (binomial tests, $n = 100$, $p < 0.001$ and $p = 0.004$ respectively) (Fig. 1). These attraction percentage towards these two aphid species, tested separately against non-infested plants, are not statistically different (χ^2 test, $p = 0.520$).

However, when plants infested with these two aphid species were tested against each other, plants infested by *Aphis fabae* attracted significantly more ant foragers (61 %, binomial test, $n = 100$, $p = 0.035$). The same tendency is also observed for honeydews of these two species, and for cultures of bacteria found in these honeydews. When presented with honeydews of the two species, 65% of ant foragers chose the *Aphis fabae* honeydew branch (binomial test, $n = 60$, $p = 0.027$). Facing a choice between cultures of *S. xylosus* and *S. sciuri*, 65% of ant foragers selected the *S. xylosus* branch (binomial test, $n = 60$, $p = 0.027$). These attraction levels towards *Aphis fabae*, its honeydew, and a bacterium from its honeydew, tested against *Acyrthosiphon pisum*, its honeydew, and a bacterium from its honeydew respectively, are statistically not different from the attractiveness observed in the first assay (χ^2 tests, $p = 0.133$, $p = 0.618$ and $p = 0.618$ respectively).

No significant differences in speed were observed between branches of the olfactometer in any assay (t-tests, equality of variances verified, all $p > 0.099$).

Characterization of honeydew's and bacterial VOCs emission profiles

Twenty-eight volatile chemicals, including esters, alcohols, acids, aldehydes and ketones, were identified from honeydew and bacterial cultures. A comparison with other data from our former investigations is shown in table 2.

Among the 28 compounds observed in honeydews, 9 were observed in honeydews of both species. However, the relative amount of these compounds may vary strongly between species. For example, benzenethanol constitutes 53% of total peak area for *Aphis fabae* honeydew VOC, and only 1.7% of total peak area for *Acyrthosiphon pisum*.

Sixteen of the 28 compounds identified from honeydews were also observed in *Staphylococcus* cultures. The mVOCs emitted by both *Staphylococcus* species are qualitatively nearly identical, the only difference being linalool solely observed for *S. xylosus*. However, strong quantitative differences are also observed (Table 2).

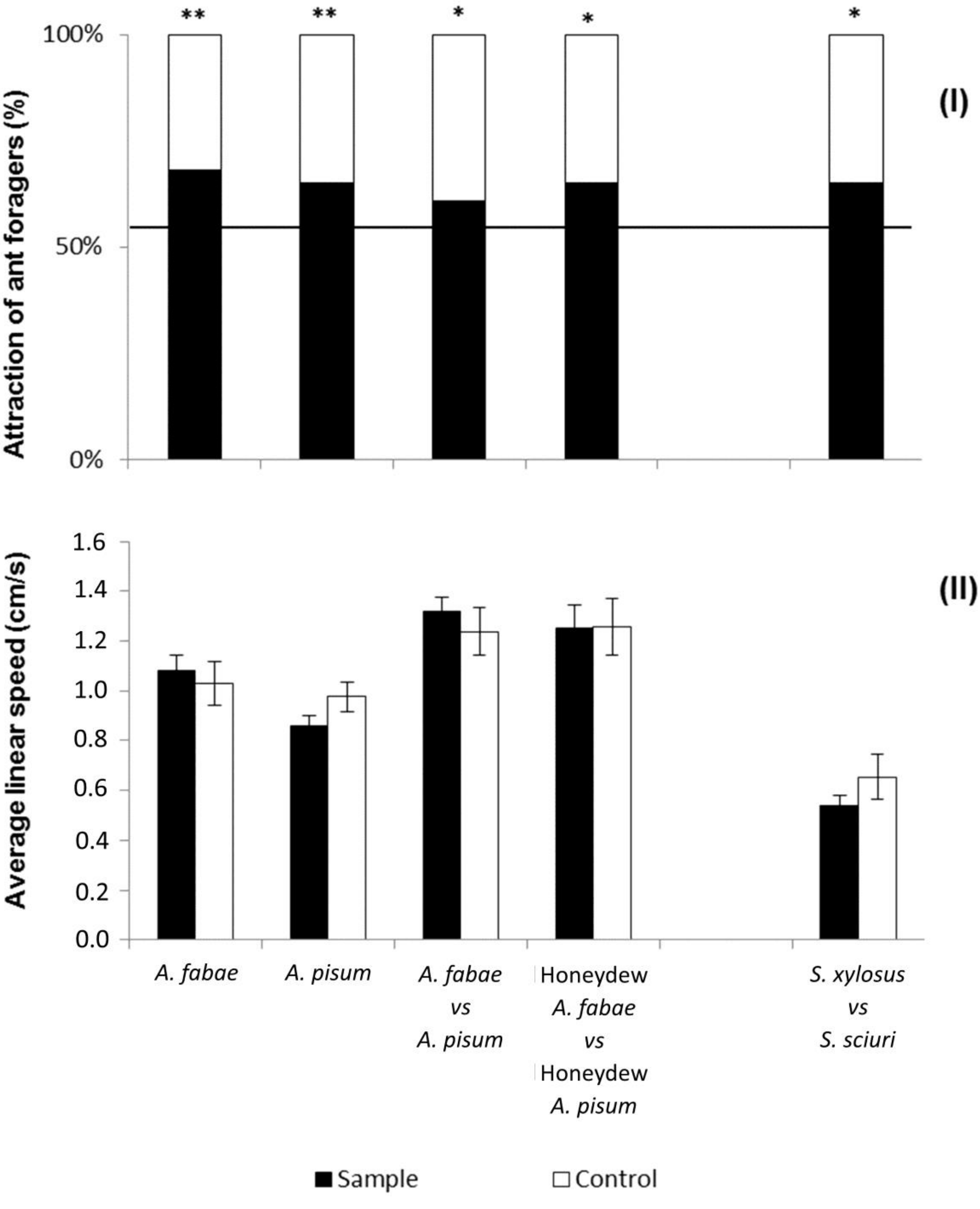


Fig. 1 - Behavioral response of *L. niger* to VOCs related to two different aphid species

Five preference tests of *L. niger* foragers in a two way-olfactometer presented with VOCs originating from (1) and (3) *Aphis fabae* infested plants (n=100), (2) *Acyrthosiphon pisum* infested plants (n=100), (4) *Aphis fabae* honeydew (n=60), (5) 863 medium inoculated with *S. xylosus* (n=60). Controls for these five treatments were (1) and (2) healthy plants, (3) *Acyrthosiphon pisum* infested plants, (4)) *Acyrthosiphon pisum* honeydew, and (5) 863 medium inoculated with *S. sciuri*. **(I)** Ants choice between sample and control branches as their final destination in the olfactometer (%). **(II)** Linear speed (cm/s, X ± SEM) of ants in each olfactometer branch. * and ** indicate significant differences from the control at P<0.05 and P<0.01, respectively.

Table 2| Volatile organic compounds (VOCs) found in aphid-secreted honeydew and bacteria-inoculated medium

Retention time (min)	VOC	*A.fabae* honeydew†	*S. xylosus*-inoculated 863 liquid medium†	*S. sciuri*-inoculated 863 liquid medium	*A. pisum* honeydew‡
1.65	Propanone	0.85±0.14	0.98±0.02	3.65±2.53	9.25 ± 2.99
1.78	Methyl acetate	6.75±3.83			
2.05	2,3-Butanedione	0.45±0.17	0.27±0.03	0.15±0.03	2.31 ± 1.26
2.22	Ethyl acetate	19.95±15.11			
2.39	2-Methylpropanol	0.67±0.25			
2.61	3-Methylbutanal		1.54±0.32	0.32±0.01	14.01 ± 3.24
2.71	2-Methylbutanal		1.91±0.02	0.57±0.01	12.92 ± 1.33
2.97	Ethanoic acid	1.47±0.82	46.36±0.73	15.73±1.02	
3.50	1-Methylethyl acetate	0.10±0.03			
3.57	3-Hydroxy-2-butanone	0.05±0.05			0.78 ± 0.24
3.70	3-Methyl-3-buten-1-ol	0.28±0.13	0.05±0.01	0.03±0.00	0.89 ± 0.39
3.80	3-Methyl-1-butanol	5.40±3.41	16.45±1.03	37.93±3.14	12.32 ± 5.58
3.87	2-Methyl-1-butanol	1.73±0.35	4.43±0.12	3.44±0.13	
4.52	2-Methylpropanoic acid		1.20±0.04	1.67±0.01	
4.57	2-Methylpropyl acetate	0.38±0.20			
5.51	Butanoic acid	0.05±0.03	5.43±0.17	1.85±0.22	6.24 ± 3.45
7.06	3-Methylbutanoic acid	1.93±1.13	7.88±0.10	22.30±0.65	4.56 ± 0.45
7.18	2-Methylbutanoic acid	0.99±0.70	0.93±0.00	5.79±0.39	6.73 ± 5.55
7.28	3-Methyl-1-butyl acetate	1.03±0.44	0.18±0.02	0.45±0.10	
7.36	2-Methyl-1-butyl acetate	0.46±0.15	< 0.01	< 0.01	
8.32	2,5-Dimethylpyrazine		1.41±0.03	0.34±0.01	0.31 ± 0.16
9.83	Benzaldehyde	0.14±0.07	5.18±0.19	3.15±0.14	
10.58	6-Methyl-5-heptene-2-one		4.00±0.49	0.58±0.09	
12.04	2-ethyl-1-hexanol	0.17±0.02			
12.42	Benzeneacetaldehyde	0.40±0.13	0.63±0.10	0.19±0.02	
14.18	Linalool		0.37±0.02		
14.56	Benzeneethanol	53.12±16.40	0.82±0.15	1.85±0.14	1.73 ± 0.50
17.81	Phenylethyl acetate	3.65±1.89			
	2-Methyl-2-buten-1-ol				14.41 ± 1.39
	3-Methyl-2-butenal				10.73 ± 2.71
	Limonene				2.81 ± 0.17

Relative proportions (%±s.e.m.; honeydew: n=4 ; cultures: n=3) of the volatile compounds collected by solid-phase microextraction and analyzed by gas chromatography-mass spectrometry

† data from Fischer *et al.* (2015a) ; ‡ data from Leroy *et al.* (2011)

Discussion

In order to establish and maintain a mutualistic relationship, ant foragers have first to find aphid colonies and asses their suitability as partners based on various parameters including aphid morphology and behavior, honeydew quality and quantity …. However, in order to asses these criteria, ant foragers must already have found the aphid colony. Previous studies already showed ants ability to orient their search for aphid colonies using volatile cues (Verheggen *et al.* 2012; Fischer *et al.* 2015a). The data presented here is the first evidence of distant aphid species discrimination by ants that is driven by aphid honeydew and mediated by the associated microflora through release of VOCs, in laboratory conditions.

Using a methodology previously applied to highlight honeydew mVOCs effect on ant behavior (Fischer *et al.* 2015a), we observed similar ant attraction toward both myrmecophilous and non-myrmecophilous aphid species when the aphids were presented alone. However, when given a choice between the two aphid species, ant showed a significant preference for the myrmecophilous *Aphis fabae* with an attraction level similar to the attraction observed for this aphid presented alone. Moreover, this tendency is supported by the relative attractiveness of honeydews and bacterial cultures issued of these two aphid species, confirming both ants ability to distantly discriminate between two aphid species, and honeydew VOCs involvement in that discrimination. Even though mVOCs emitted from honeydew have been reported to attract aphid enemies and potential ant partners (Leroy et al. 2011a; Fischer et al. 2015a), this is the first evidence of ant ability to use such volatile cues to distantly discriminate two aphid species. Furthermore, the attractiveness shown by *Acyrthosiphon pisum* when presented alone, strongly reduced when *Aphis fabae* is added in the possible choices, suggests that ants might adapt their response depending on available resources.

Foraging behavior of ant foragers is influenced by the colony needs, depending on various parameters including population, brood presence … (Portha *et al.* 2004; Buffin *et al.* 2011; Oliver *et al.* 2012). For example, in laboratory conditions, ant larval presence significantly reduces the growth rate of tended aphid colonies, suggesting that ant colonies balance the flow of two separate resources from aphid colonies, renewable sugars or a protein-rich meal, depending on demand from ant larvae within the nest (Oliver *et al.* 2012). In this context, ant ability to distantly recognize aphid species and discriminate between potential mutualistic partners, providing both a stable sugar source and potential preys, and non-myrmecophilous species, constituting only preys, would be an advantage.

The volatile compounds released by both aphid honeydews and bacterial cultures are qualitatively very close. They originate mostly from bacterial degradation of diverse compounds. Potential origins and biosynthetic pathways of each compounds, as well as their involvement in different ant species communication, have been previously discussed (Fischer *et al.* 2015a). Although qualitative VOCs profiles detected are very close, even nearly identical for the two tested bacterial cultures, ants still shown a preference for *Aphis fabae*-related samples, suggesting recognition based on ratios between compounds instead of the attractiveness of a single molecule or group of molecules. The impact of compounds ratios on insects communication is a well-known phenomenon which has already been highlighted for numerous insect species, including ant and aphid species (Castracani *et al.* 2008; Cardé and Millar 2009; Byers *et al.* 2013; Han *et al.* 2014).

Honeydew VOCs seem to play a role in distant aphid discrimination, and their microbial origins are interesting. Indeed, this signal depends on microbial populations growing in aphid gut and honeydew, which is shaped by various parameters among which microbe's ability to survive in that kind of hostile environment (low oxygen, high osmotic pressure, potential antimicrobial compounds…) and affinity for the host constitute key factors (González-Teuber *et al.* 2009; Herrera *et al.* 2010; Álvarez-Pérez *et al.* 2012; Kirzinger and Stavrinides 2012). Thus, while honeydew composition and potential aphid antimicrobial secretion contribute to select microbial populations and modulate their emissions, they constitute an aphid-presence signal mostly independent from the aphid itself, which is thus less likely to be altered by the aphid (mimicry, dissimulation…). It constitutes a reliable "honest" cue for ant foragers.

The data presented here indicate that, in addition to attraction towards potential food sources, honeydew microbial volatile compounds enable ant foragers to distantly discriminate between aphid species. This underlines once more the key role of honeydew in ant-aphid interactions, adding distant discrimination in its already multiple known effects on this mutualism. Moreover, these results emphasize the importance of investigating the presence and potential effects of microbes in insect symbioses.

Acknowledgement

Christophe Fischer is financially supported by a PhD grant from the *Fonds pour la formation à la Recherche dans l'Industrie et l'Agriculture* (FRIA). This project is also financially supported by a *Fonds de la Recherche Fondamentale Collective* (F.R.F.C. – F.N.R.S.) research project (2.4600.09)

Disclosure

The authors declare no conflicts of interest, or specific financial interests, relationships or affiliations.

References

Álvarez-Pérez S, Herrera CM, de Vega C (2012) Zooming-in on floral nectar: A first exploration of nectar-associated bacteria in wild plant communities. FEMS Microbiol Ecol 80:591–602. doi: 10.1111/j.1574-6941.2012.01329.x 10.1111/j.1365-294X.2011.05402.x

Bristow CM (1991) Why are so few aphids ant-tended? In: Cutler DF, Huxley C (eds) Ant/Plant Interactions. Oxford University Press, pp 104–119

Buffin A, Mailleux AC, Detrain C, Deneubourg JL (2011) Trophallaxis in Lasius niger: A variable frequency and constant duration for three food types. Insectes Soc 58:177–183. doi: 10.1007/s00040-010-0133-y

Byers JA, Fefer D, Levi-Zada A (2013) Sex pheromone component ratios and mating isolation among three Lygus plant bug species of North America. Naturwissenschaften 100:1115–1123. doi: 10.1007/s00114-013-1113-7

Cardé RT, Millar JG (2009) Chapter 195 - Pheromones. In: Cardé VHRTBT-E of I (Second E (ed) Encyclopedia of Insects. Academic Press, San Diego, pp 766–772

Castracani C, Tamarri V, Grasso DA, et al (2008) Chemical communication in mating behaviour of the slave-making ant Polyergus rufescens (Hymenoptera, Formicidae): 3-Ethyl-4-methylpentanol as a critical component of the queen sex pheromone. Insectes Soc 55:137–143. doi: 10.1007/s00040-008-0981-x

Dixon AFG (1985) Aphid ecology. Blackie ; Distributed in the U.S.A. by Chapman and Hall, Glasgow

El-Ziady S, Kennedy JS (1956) Beneficial effects of the common garden ant, Lasius niger L., on the black bean aphid, Aphis fabae Scopoli. Proc R Ent Soc Lond 31:5.

Fischer CY, Lognay GC, Detrain C, et al (2015a) Bacteria may enhance species association in an ant–aphid mutualistic relationship. Chemoecology 1–10. doi: 10.1007/s00049-015-0188-3

Fischer CY, Vanderplanck M, Lognay GC, et al (2015b) Do aphids actively search for ant partners? Insect Sci 22:283–288. doi: 10.1111/1744-7917.12125

Francis F, Vandermoten S, Verheggen F, et al (2005) Is the (E)-β-farnesene only volatile terpenoid in aphids? J Appl Entomol 129:6–11.

González-Teuber M, Eilmus S, Muck A, et al (2009) Pathogenesis-related proteins protect extrafloral nectar from microbial infestation. Plant J 58:464–473.

Guénard B, Lucas É, Francoeur A (2007) Mutualisme fourmis pucerons et guilde aphidiphage associée : le cas de la prédation furtive. Université du Québec à Montréal

Han B-Y, Wang M-X, Zheng Y-C, et al (2014) Sex pheromone of the tea aphid, Toxoptera aurantii (Boyer de Fonscolombe) (Hemiptera: Aphididae). Chemoecology 24:179–187. doi: 10.1007/s00049-014-0161-6

Herrera CM, Canto A, Pozo MI, Bazaga P (2010) Inhospitable sweetness: Nectar filtering of pollinator-borne inocula leads to impoverished, phylogenetically clustered yeast communities. Proc R Soc B Biol Sci 277:747–754. doi: 10.1098/rspb.2009.1485 10.1034/j.1600-0706.2000.910301.x

Holman J (2009) The Aphids and their Host Plants. In: Host Plant Catalog of Aphids. Springer Netherlands, pp 7–651

Kirzinger MWB, Stavrinides J (2012) Host specificity determinants as a genetic continuum. Trends Microbiol 20:88–93. doi: http://dx.doi.org/10.1016/j.tim.2011.11.006

Leroy PD, Sabri A, Heuskin S, et al (2011) Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. Nat Commun 2:7. doi: Artn 348 Doi 10.1038/Ncomms1347

Oliver TH, Leather SR, Cook JM (2012) Ant larval demand reduces aphid colony growth rates in an ant-aphid interaction. Insects 3:120–130. doi: 10.3390/insects3010120

Portha S, Deneubourg JL, Detrain C (2004) How food type and brood influence foraging decisions of Lasius niger scouts. Anim Behav 68:115–122. doi: DOI 10.1016/j.anbehav.2003.10.016

Stadler B (1997) The relative importance of host plants, natural enemies and ants in the evolution of life-history characters in aphids. In: Dettner K, Bauer G, Völkl W (eds) Vertical food web interactions : evolutionary patterns and driving forces. Springer - Verlag, pp 241–256

Stadler B, Dixon AFG (2005) Ecology and evolution of aphid-ant interactions. Annu Rev Ecol Evol Syst 36:345–372.

Stadler B, Dixon AFG (1999) Ant attendance in aphids: why different degrees of myrmecophily? Ecol Entomol 24:363–369.

Verheggen FJ, Diez L, Sablon L, et al (2012) Aphid Alarm Pheromone as a Cue for Ants to Locate Aphid Partners. PLoS One 7:e41841. doi: 10.1371/journal.pone.0041841

Wilson EO (1955) A Monographic Revision of the Ant Genus Lasius. Bull. Mus. Comp. Zool. 113:59–77.

CHAPITRE VI

RECHERCHE D'UN PARTENAIRE MUTUALISTE PAR LE PUCERON

S'il est généralement admis que les fourmis jouent un rôle actif dans la recherche de pucerons mutualistes, l'hypothèse inverse est plus rarement envisagée et, à notre meilleure connaissance, n'a pas fait l'objet d'investigations expérimentales. Néanmoins, une plus forte concentration en pucerons est parfois rencontrée à proximité de fourmilières, d'où la question déjà posée par certains auteurs : les pucerons ailés s'établissent-ils préférentiellement à proximité de fourmilières lorsqu'ils se dispersent, ou survivent-ils simplement mieux en présence de leur protecteur ? (Hopkins and Thacker 1999). Cette question sera développée dans ce chapitre, en s'intéressant d'une part au potentiel effet attractif lié à la présence de fourmis dans le choix d'une plante hôte par un puceron ailé, et d'autre part à l'effet de rétention lié à la présence de fourmis une fois le puceron ailé sur la plante.

DO APHIDS ACTIVELY SEARCH FOR ANT PARTNERS?

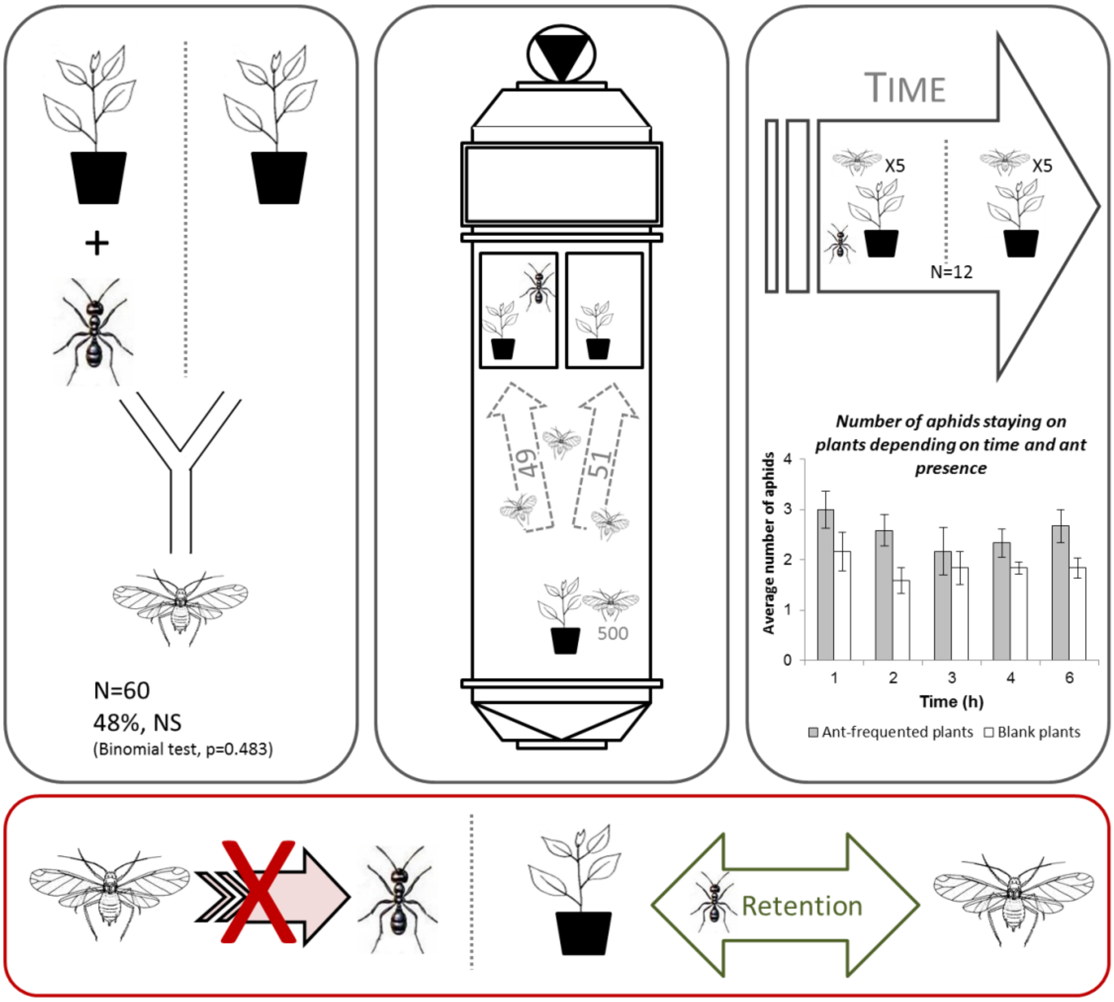
Christophe Y. Fischer[1], Maryse Vanderplanck[2], Georges C. Lognay[1], Claire Detrain[3] and François J. Verheggen[4]

[1] Laboratory of Analytical Chemistry, University of Liege, Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium. (christophe.fischer@ulg.ac.be; georges.lognay@ulg.ac.be)

[2] Research Institute of Biosciences, Laboratory of Zoology, University of Mons, Place du Parc 20, 7000 Mons, Belgium. (maryse.vanderplanck@umons.ac.be)

[3] Unit of Social Ecology, Université Libre de Bruxelles, CP231,50 avenue F. Roosevelt, B-1050 Brussels, Belgium. (cdetrain@ulb.ac.be)

[4] Department of Functional and Evolutionary Entomology, University of Liege, Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium. (fverheggen@ulg.ac.be)



Graphical abstract - Do aphids actively search for ant partners?

Abstract - The aphid-ant mutualistic relationships are not necessarily obligate for neither partners but evidence is that such interactions provide them strong advantages in terms of global fitness. While it is largely assumed that ants actively search for their mutualistic partners namely using volatile cues; whether winged aphids (*i.e.* aphids' most mobile form) are able to select ant-frequented areas had not been investigated so far. Ant-frequented sites would indeed offer several advantages for these aphids including a lower predation pressure through ant presence and enhanced chances of establishing mutuaslistic interactions with neighbour ant colonies. In the field, aphid colonies are often observed in higher densities around ant nests, which is probably linked to a better survival ensured by ants' services. Nevertheless, this could also result from a preferential establishment of winged aphids in ant-frequented areas. We tested this last hypothesis through different ethological assays and show that the facultative myrmecophilous black bean aphid, *Aphis fabae* L., does not orientate its search for a host plant preferentially towards ant-frequented plants. However our results suggest that ants reduce the number of winged aphids leaving the newly colonized plant. Thus, ants involved in facultative myrmecophilous interactions with aphids appear to contribute to structure aphid populations in the field by ensuring a better establishment and survival of newly established colonies rather than by inducing a deliberate plant selection by aphid partners based on the proximity of ant colonies.

Keywords: ant, aphid, *Aphis fabae*, *Lasius niger*, mutualism, partner research

Citation: *Fischer CY, Vanderplanck M, Lognay GC, Detrain C and Verheggen F (2015) Do aphids actively search for ant partners? Insect Sci 22:283–288. doi: 10.1111/1744-7917.12125*

Introduction

The ant-aphid mutualism is one of the most studied animal relationships (Verheggen *et al.* 2012). While aphids provide ants with a stable and reliable food source (i.e. sugar-rich honeydew), ants improve the overall hygiene of the tended aphid colony and protect it against various natural enemies (Way 1963; Stadler and Dixon 2005). This relationship, which can be obligate or facultative depending on the aphid species considered, provides both partners with strong advantages in terms of global fitness. Therefore ants and aphids would benefit from behavior favoring the establishment of a mutualism (Verheggen et al. 2009a). Mechanisms enhancing encounters between ants and aphids are thus key elements in the success of such partnerships.

Ants are known to actively search for aphid partners namely by using volatiles cues like the aphid alarm pheromone (E-β-farnesene, constantly released at low levels by aphid colonies) to distantly orientate their search (Verheggen *et al.* 2012). Once a potential aphid partner is detected, its suitability for a partnership is then assessed based on multiple criteria including aphid behavior, aphid morphological adaptation for ant-tending and overall honeydew quality and quantity (Way 1963; Fischer *et al.* 2001; Stadler and Dixon 2005; Detrain *et al.* 2010; Lang and Menzel 2011).

Small-sized aphid colonies such as newly established ones, experience proportionally the highest level of ant tending (Breton and Addicott 1992). While presenting higher costs in terms of honeydew production for the aphids, this also increases the protection offered by ants, which would become particularly profitable for aphids under high predation pressure (Breton and Addicott 1992; Yao *et al.* 2000; Yoo and Holway 2011). When the aphid colony becomes too large, ants invest less in tending behavior whereas they start preying on aphids, thereby reducing aphid benefits per capita (Breton and Addicott 1992; Sakata 1995; Sakata 1999).

Ants are also known to structure aphid colonies in the field so that myrmecophilous aphid species are frequently observed at higher densities in the surroundings of ant colonies (Seibert 1992; Hopkins and Thacker 1999; Bishop and Bristow 2003; Stewart-Jones *et al.* 2008; Minarro *et al.* 2010). Since ants provide myrmecophilous aphids with protected areas where both enemies and interspecific competition pressure are reduced (non mutualistic species being considered as preys) (Fischer *et al.* 2001; Bishop and Bristow 2003; Stewart-Jones *et al.* 2008; Minarro *et al.* 2010) ; one can assume that winged aphids, the dispersal morph of most aphids, would find benefits in selecting host plants in such ant-frequented areas. However, whether such an active search for ants' proximity occurs in aphids has not been considered so far.

In this paper, we investigate the impact of ant occurrence on the host plant selection by winged aphids as well as on the behavior of aphids newly arrived on a host plant.

Material and methods

Study model

We selected the facultative myrmecophilous black bean aphid, *Aphis fabae* (Scopoli) and the black garden ant *Lasius niger* L. as biological models, these insects being common mutualistic partners. For our bioassays, both colonies of ants and aphids were collected in the surroundings of Gembloux (Belgium).

The two species were reared in a climate-controlled room (16 h light-8 h dark photoperiod; 20 ± 2 °C (± SD)). Aphid colonies were fed on broad beans (*Vicia faba* L.) cultivated in a mix of perlite and vermiculite (1/1) for several generations. Ant nests collected in the field were placed in plastic containers coated with polytetrafluoroethylene (Fluon®,Whitford, U.K.) to avoid escape. Six nests were used during the assays. Laboratory rearing nests consisted in test tubes covered with a red transparent foil and hosted a queen, brood and a minimum of 500 individuals. Dead insects were given weekly (flies, mealworms) in quantities consumed within two days. Both water and aqueous brown sugar solution (342 g/L) were provided *ad libitum* using dispenser made with a glass tube filled with water or sugar solution and closed with a cotton wool plug.

Ethological assays

Since winged individuals are the dispersal form of aphids and the most prone to establish new colonies away from the initial one, only this morph has been studied in the following bioassays. Two sets of assays have been developed: Y olfactometry and plant choice in wind tunnel.

Two-ways olfactometry

The attractiveness of ant-visited plants for winged *A. fabae* has been assessed using a Y shaped glass olfactometer (internal diameter: 0.5 cm; 7 cm long branches). All assays have been conducted at 22 ± 2 °C in a dark-walled chamber presenting no visual cues that might influence aphid choices. Samples and controls were placed in 4 L glass jars. Air purified on charcoal filters was pushed into those jars at 300 mL/min and then in the olfactometer's branches. The sample consisted in a pot of nine *V. faba* plants and one nest tube containing 100 ants placed with the plants for two days. The control contained only "blank" plants -i.e. without ants. Sixty winged aphids were collected on heavily infested plants using a soft brush and placed one by one in the olfactometer. Each test ended when aphids, tested individualy, reached the end of a branch. For each aphid, the chosen branch and the time needed to complete the assay were recorded.

Wind tunnel

A second choice test has been performed in a wind tunnel made of Plexiglas (2.4 x 0.8 x 0.6 m). At one side of the wind tunnel, two plastic containers (0.56 x 0.36 x 0.11 m) wall-coated with Fluon® (Whitford Plastics Ltd., UK) were placed side-by-side (Fig. 1). A pot of nine *V. faba* was placed at the center of each plate and one ant colony was added in one of the plates two days prior to the beginning of the assay. The Fluon coating prevented ant escape and ensured that aphids could reach the plants only by flight. At the other side of the tunnel (at 180 cm from the aforesaid plants), 250 winged aphids were introduced on a plant heavily infested by apterous aphids in order to favor their dispersal flight. The experimental conditions were as follows: velocity of 0.4 m/s (laminar flow); T = 20 ± 2 °C; RH = 60–70 %; illumination = 2.300 lux. After 24 h, newly arrived aphids were counted on each healthy plant. Preliminary experiments have shown that this delay period was a good compromise to optimize the number of aphids reaching the plant and to limit its colonization by the offspring of newly arrived aphids. This assay (aphids counting) has been run in two replicates.

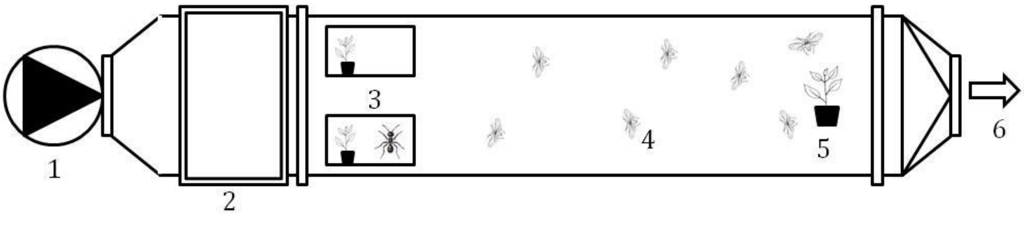


Figure 1 | Wind tunnel used to investigate *Lasius niger* impact on *Aphis fabae* alate choice of host plant

Experimental setup of wind tunnel used to investigate the impact of ant presence on the choice of host plant by aphids. 1: Fan; 2: Filters; 3: plastic plates containing *V. faba* frequented by *L. niger* and control *V. faba*; 4: Winged *A. fabae*; 5: Winged *A. fabae* release point (highly infested plant); 6: Wind direction.

Retention effect of ants

The effect of ant presence on the retention of newly arrived winged *A. fabae* on a plant was investigated in a third laboratory assay. Pots of nine *V. faba* were infested by groups of five winged aphids. These infested plants were placed either in presence of an ant colony, so ants could freely access the plants, or absence of ants. The aphids remaining on the plant were counted after 1, 2, 3, 4 and 6 hours. The tests were run at 22 ± 2 °C in 3.2 x 3 m greenhouse chambers. Four pots were placed per chambers at the corner of a 1.5m square at the center of the room. The two conditions were tested in separate chambers. Twelve pots were analyzed (aphids counting after 1, 2, 3, 4 and 6 hours) for each experimental condition.

Statistical analyses

We performed a binomial test to detect significant differences in proportions for the Y-olfactometer assay. The average linear speeds recorded for the two branches of the Y-olfactometer were compared using Student t-tests (normality and homoscedasticity assumptions met, tested with Ryan-Joiner and F-Test respectively). For the aphid retention assay, the significance of the effect of time and ant presence was assessed using a repeated measures analysis of variance (ANOVA) with time (1, 2, 3, 4 and 6 hours) as within-subject factor, and treatment (ant presence versus ant absence) as between-subject factor. Variables were assessed for distributional properties and degrees of freedom, and were adjusted with Greenhouse-Geisser correction when sphericity assumption was violated (i.e. Mauchly's test with p-value less than 0.05). Repeated measures ANOVA was performed with R 3.0.2 (R Core Team, 2013); other analyses were computed with Minitab 15.1 (State College, Pennsylvania, USA). Significance was set at $p < 0.05$.

Results

Two-ways olfactometry

Among sixty aphids tested in two-ways olfactometer, 29 individuals chose the ant-visited plant against 31 for the blank plant. Every tested aphid completed the assay and no choice changes were observed during the assays. The attractiveness of the sample and the control are not significantly different (binomial tests, p-value = 0.483). Average times spent in the olfactometer while going on the ant-visited plant branch or the control branch are

387 ± 212 s and 340 ± 220 s, respectively and do not significantly differ (t-test, p-value = 0.403).

Wind tunnel

In a wind tunnel, when facing the choice between ant-frequented plants and control plants, winged *A. fabae* did not seem to mark any preference. Among the 500 winged aphids released in the wind tunnel, one fifth flew to one of the two tested plants placed on the other side of the wind tunnel, with 49 and 51 aphids landing on the ant-frequented plants and the control plants, respectively.

Retention effect of ants

As expected, there is a main effect of ant presence as it results in a greater aphids retention (repeated measures ANOVA, p = 0.037), with a non significant time × treatment interaction (repeated measures ANOVA with Greenhouse-Geisser correction, p = 0.583) (Fig. 2). The time elapsed since aphids release does not significantly influence the number of aphids remaining on plants (repeated measures ANOVA with Greenhouse-Geisser correction, p = 0.537) (Fig. 2). The greatest number of aphids leaves the plant within the first hour following their release. Then, the number of aphids remaining on plants remains quite stable and higher on ant-frequented plants (Fig. 2). Ants thus seem to limit aphid dispersal far from the infested plant and to stabilize their partner in their initial location.

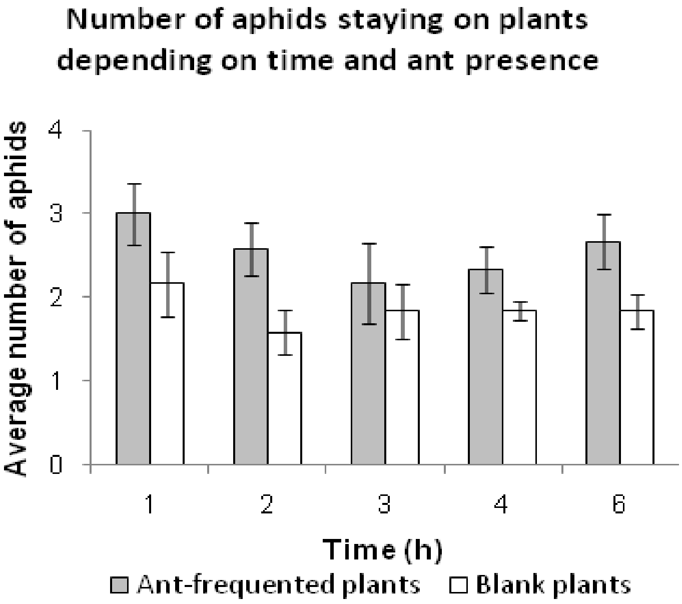


Figure 2 | Average number of aphids staying on plants depending on time and ant presence
Average numbers of aphids left on a plant at different times after the release of five winged aphids on the plant (Mean ± SEM). N=12.

Discussion

In the field, higher densities of myrmecophilous aphids colonies are observed in the surroundings of ant nests (Seibert 1992; Hopkins and Thacker 1999). Ants' presence enhance aphid survival, especially under a strong predation pressure, offering them diverse services including cleaning and protection, which can explain these observations. Whether this phenomenon is reinforced by the preferential establishment of winged aphids in ant-frequented areas had not yet been studied. The selection of such favorable sites, by an active orientation towards the area or by an enhanced retention on such ant-frequented plants, could indeed constitute an advantage for winged aphids, the dispersal morph of aphids, and for their offspring.

Our results show no significant impact of ant presence on the distant orientation of winged *A. fabae* toward host plants. Indeed, both two-ways olfactometry and wind tunnel assays showed no choice preference for an ant-frequented plant. Moreover, the attraction level of aphids expressed by their average linear speed in olfactometer was similar in the two branches of the system. In another assay in greenhouse under semi-natural conditions undertaken to support the results, only 1% of the 1000 winged aphids (500 aphids, replicated twice) released in the center of the room reached the plants placed either side of the chamber (C.Y. Fischer, unpublished data). In contrast with previous studies (Pickett *et al.* 1992), this suggests that *A. fabae* attraction towards host plants is not very efficient in case of isolated plants under these conditions. For other aphid species, like *Cryptomyzus korschelti* (Börner), host plant volatiles induce a positive upwind anemotaxis (Pickett *et al.* 1992). Thus, the absence of wind in the greenhouse assay might partially explain the low number of *A. fabae* aphids reaching plants. Nevertheless, in the wind tunnel assay, the proportion of aphids reaching their host plant was also relatively low, most of them making short erratic flights around their release point and struggling against the wind. These two assays confirm bad flight abilities of the black bean aphid (Dixon and Howard 1986; Goldansaz and McNeil 2006). Being bad flyers, winged aphids would then benefit from clinging to a favorable site when they reach it.

Ant presence can have an impact on aphid distribution in the field by providing them with enemy- and competition-reduced spaces (Fischer *et al.* 2001; Bishop and Bristow 2003; Stewart-Jones *et al.* 2008; Minarro *et al.* 2010). The extent of that protection depends on the ant species, its aggressiveness ant territoriality, its affinity for the aphid partner, the intensity of the tending and the enemy considered (El-Ziady and Kennedy 1956; Way 1963; Stadler and Dixon 2005; Guénard et al. 2007; Verheggen et al. 2009a; Novgorodova and Gavrilyuk 2012). For example, *Harmonia axyridis* (Pallas) adults and larvae are quickly attacked and removed from the plant (Herbert and Horn 2008), while other enemies, like the parasitoid *Lysiphlebus cardui* (Marshall) or the cecidomyid predator *Aphidoletes aphidimyza* (Rondani), are able to prey freely on aphids without being disturbed by ants (Liepert and Dettner 1996; Guénard *et al.* 2007). Moreover, ants play a role in structuring aphid populations, favoring their preferred species and preying on the others (Fischer *et al.* 2001; Bishop and Bristow 2003; Stewart-Jones *et al.* 2008; Minarro *et al.* 2010). They are also known to limit aphid dispersal in well-established tended colonies by reducing winged aphids production and affecting their flight abilities (Kleinjan and Mittler 1975; Oliver *et al.* 2007; Yao 2012). Our results bring one more possible structuring effect of ants on aphid population. Indeed, while winged *A. fabae* are not attracted toward ant-frequented areas, they tend to stay more on ant-frequented plants, and are thus more likely to start new colonies on these plants. Once detected by ants, newly arrived aphids and their first born are thus likely to experience high levels of attendance and the related hygienic and protection benefits. However, *A. fabae* is a facultative myrmecophile and is thus able to survive without ant tending. It might be interesting to investigate whether obligate myrmecophilous species show the same behavior.

Within ant-aphid mutualism, ant seems thus to be the only one actively searching for partnership. Our results show no attraction of winged *A. fabae* by ant presence, while ants are known to orientate their scouting based on aphid-linked volatile organic compounds (Verheggen *et al.* 2012). Nevertheless, winged aphids stay significantly more on ant-frequented plants. Thus, the higher prevalence of aphid colonies in the surroundings of ant nests observed in the field, primarily linked to a better aphid survival through protection and reduction of competition, does not seem related to a deliberated flight orientation of winged aphids towards the ant partner, but could be influenced by a better retention of winged aphids on ant-frequented plants.

Acknowledgement

Christophe Fischer is financially supported by a PhD grant from the *Fonds pour la formation à la Recherche dans l'Industrie et l'Agriculture* (FRIA). Maryse Vanderplanck received financial support from the Belgian Fund for Scientific Research (FRS-FNRS). This project is also financially supported by a *Fonds de la Recherche Fondamentale Collective* (F.R.F.C. – F.N.R.S.) research project (2.4600.09).

References

Bishop DB, Bristow CM (2003) Effects of the presence of the Allegheny mound ant (Hymenoptera : Formicidae) in providing enemy-free space to myrmecophilous aphid and soft scale populations. Ann Entomol Soc Am 96:202–210.

Breton LM, Addicott JF (1992) Density-Dependent Mutualism in an Aphid-Ant Interaction. Ecology 73:2175–2180.

Detrain C, Verheggen FJ, Diez L, et al (2010) Aphid-ant mutualism: how honeydew sugars influence the behaviour of ant scouts. Physiol Entomol 35:168–174.

Dixon AFG, Howard MT (1986) Dispersal in Aphids, A Problem in Resource Allocation. In: Danthanarayana W (ed) Insect Flight. Springer Berlin Heidelberg, pp 145–151

El-Ziady S, Kennedy JS (1956) Beneficial effects of the common garden ant, Lasius niger L., on the black bean aphid, Aphis fabae Scopoli. Proc R Ent Soc Lond 31:5.

Fischer MK, Hoffmann KH, Völkl W (2001) Competition for mutualists in an ant-homopteran interaction mediated by hierarchies of ant attendance. Oikos 92:531–541.

Goldansaz SH, McNeil JN (2006) Effect of wind speed on the pheromone-mediated behavior of sexual morphs of the potato aphid, Macrosiphum euphorbiae (Thomas) under laboratory and field conditions. J Chem Ecol 32:1719–1729.

Guénard B, Lucas É, Francoeur A (2007) Mutualisme fourmis pucerons et guilde aphidiphage associée : le cas de la prédation furtive. Université du Québec à Montréal

Herbert JJ, Horn DJ (2008) Effect of Ant Attendance by Monomorium minimum (Buckley) (Hymenoptera: Formicidae) on Predation and Parasitism of the Soybean Aphid Aphis glycines Matsumura (Hemiptera: Aphididae). Environ Entomol 37:1258–1263.

Hopkins GW, Thacker JI (1999) Ants and habitat specificity in aphids. J Insect Conserv 3:25–31.

Kleinjan JE, Mittler TE (1975) A chemical influence of ants on wing development in aphids. Entomol Exp Appl 18:384–388. doi: 10.1111/j.1570-7458.1975.tb00411.x

Lang C, Menzel F (2011) Lasius niger ants discriminate aphids based on their cuticular hydrocarbons. Anim Behav 82:1245–1254. doi: http://dx.doi.org/10.1016/j.anbehav.2011.08.020

Liepert C, Dettner K (1996) Role of cuticular hydrocarbons of aphid parasitoids in their relationship to aphid-attending ants. J Chem Ecol 22:695–707.

Minarro M, Fernandez-Mata G, Medina P (2010) Role of ants in structuring the aphid community on apple. Ecol Entomol 35:206–215. doi: DOI 10.1111/j.1365-2311.2010.01173.x

Novgorodova TA, Gavrilyuk A V (2012) The degree of protection different ants (Hymenoptera: Formicidae) provide aphids (Hemiptera: Aphididae) against aphidophages. Eur J Entomol 109:187–196.

Oliver TH, Mashanova A, Leather SR, et al (2007) Ant semiochemicals limit apterous aphid dispersal. Proc Biol Sci 274:3127–3131. doi: 10.1098/rspb.2007.1251

Pickett JA, Wadhams LJ, Woodcock CM, Hardie J (1992) The chemical ecology of aphids. Annu Rev Entomol 67–90.

Sakata H (1995) Density-dependent predation of the ant Lasius niger (Hymenoptera: Formicidae) on two attended aphids Lachnus tropicalis and Myzocallis kuricola (Homoptera: Aphididae). Res Popul Ecol (Kyoto) 37:159–164.

Sakata H (1999) Indirect interactions between two aphid species in relation to ant attendance. Ecol Res 14:329–340.

Seibert TF (1992) Mutualistic Interactions of the Aphid Lachnus-Allegheniensis (Homoptera, Aphididae) and Its Tending Ant Formica-Obscuripes (Hymenoptera, Formicidae). Ann Entomol Soc Am 85:173–178.

Stadler B, Dixon AFG (2005) Ecology and evolution of aphid-ant interactions. Annu Rev Ecol Evol Syst 36:345–372.

Stewart-Jones A, Pope TW, Fitzgerald JD, Poppy GM (2008) The effect of ant attendance on the success of rosy apple aphid populations, natural enemy abundance and apple damage in orchards. Agric For Entomol 10:37–43. doi: DOI 10.1111/j.1461-9563.2007.00353.x

Verheggen F, Diez L, Detrain C, Haubruge E (2009) Aphid-ant mutualism: an outdoor study of the benefits for /i Aphis fabae. Biotechnol Agron Soc Environ 13:235–242.

Verheggen FJ, Diez L, Sablon L, et al (2012) Aphid Alarm Pheromone as a Cue for Ants to Locate Aphid Partners. PLoS One 7:e41841. doi: 10.1371/journal.pone.0041841

Way MJ (1963) Mutualism between ants and honeydew-producing Homoptera. Annu Rev Entomol 8:307–344.

Yao I (2012) Ant attendance reduces flight muscle and wing size in the aphid Tuberculatus quercicola. Biol Lett 8:624–627. doi: 10.1098/rsbl.2012.0014 10.1034/j.1600-0706.2000.890101.x)

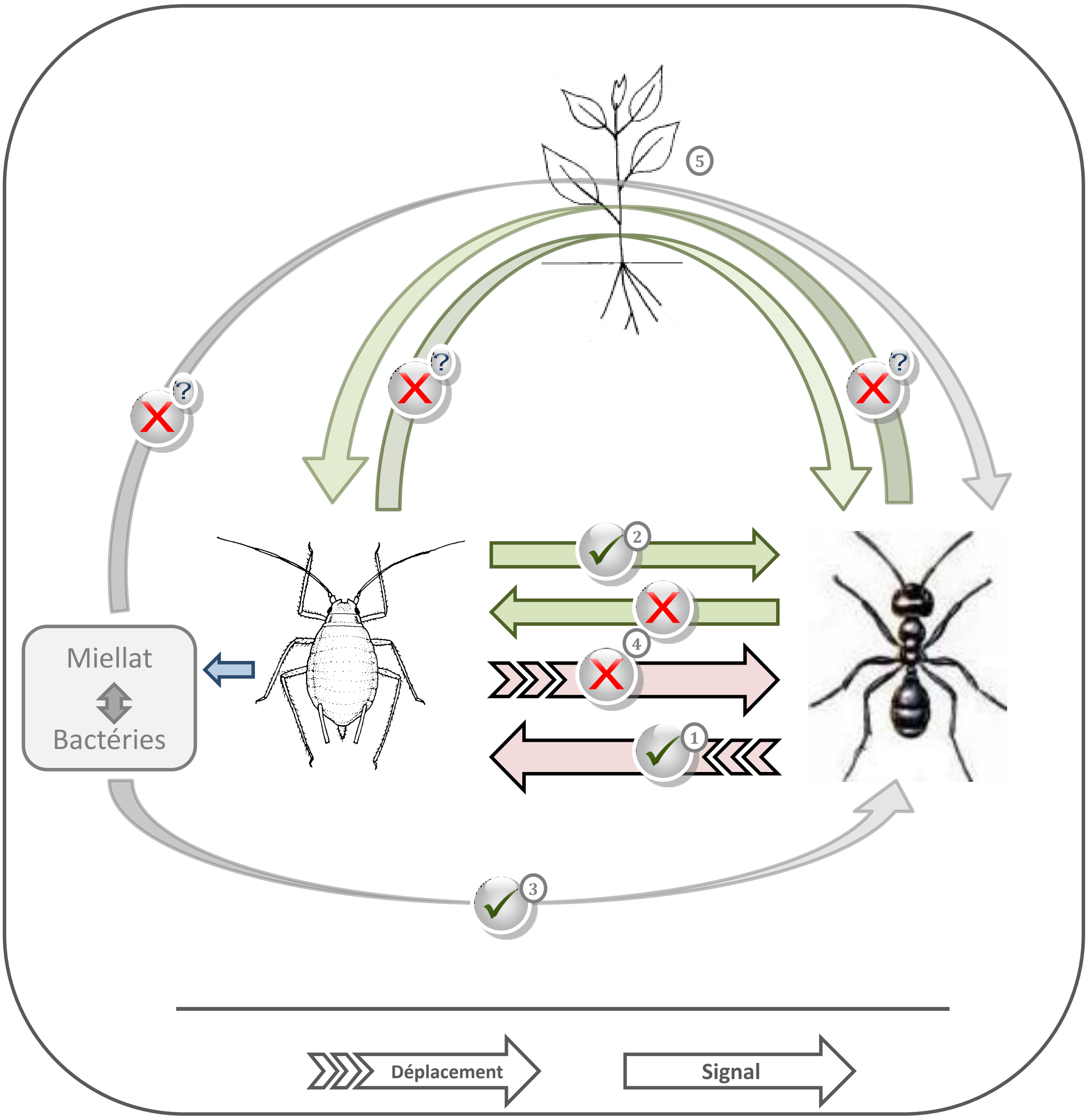
Yao I, Shibao H, Akimato S (2000) Costs and benefits of ant attendance to the drepanosiphid aphid Tuberculatus quercicola. Oikos 89:3–10.

Yoo HJS, Holway DA (2011) Context-dependence in an ant-aphid mutualism: direct effects of tending intensity on aphid performance. Ecol Entomol 36:450–458. doi: DOI 10.1111/j.1365-2311.2011.01288.x

CHAPITRE VII

CONCLUSIONS GÉNÉRALES ET PERSPECTIVES

CONCLUSIONS GÉNÉRALES ET PERSPECTIVES



Conclusion générale – Les fourmis cherchent activement leur partenaire ① en s'appuyant sur des composés volatils qu'il émet directement, tels que l'EBF ②, ou indirectement via la microflore présente dans le miellat excrété ③. Les pucerons ne semblent pas activement rechercher leur partenaire ④, mais sont stabilisés sur la plante hôte par sa présence. Aucune influence de la plante hôte en lien avec la présence de l'un ou l'autre partenaire n'a été observée ⑤.

Au cours de ce travail, le rôle des composés volatils dans la perception et la recherche d'un partenaire mutualiste a donc été mis en lumière sous divers angles. Durant les tests éthologiques menés, une attention particulière a été portée à utiliser autant que possible des doses de sémiochimiques représentatives de la réalité et obtenues de sources naturelles. La concentration des sémiochimiques peut en effet fortement influencer la réponse des insectes. Ce phénomène est souligné par certains auteurs, insistant sur l'importance de l'utilisation de doses « naturelles » lors de tests éthologiques et argumentant sur des résultats de laboratoire obtenus avec des doses massives de composés de synthèse. Ces surdosages peuvent en effet conduire à des résultats intéressants d'un point de vue pratique, utilisables par exemple dans le cadre de la gestion intégrée des ravageurs, mais non représentatifs des comportements observés aux doses réellement émises en situation naturelle et de ce fait non pertinents du point de vue écologique (Joachim et al. 2015; Joachim and Weisser 2015; Vosteen et al. 2016).

Cette approche originale d'un modèle biologique déjà bien documenté a permis de répondre aux questions initialement posées. L'ensemble des travaux publiés conduit à des conclusions complémentaires les unes aux autres, résumées dans la synthèse graphique ci-dessus. En effet, sur base du modèle biologique formé par le couple *Lasius niger* – *Aphis fabae*, il a été démontré que la fourmi mutualiste est capable de percevoir des signaux chimiques liés à la présence de pucerons et de s'en aider dans la recherche d'un partenaire mutualiste potentiel ①. Ces composés, soit directement produits par le puceron ②, soit induits par sa présence ③, sont de diverses natures et origines.

Principal composé volatil émis par le puceron *sensu stricto*, le E-β-farnésène (EBF) est un terpène régulièrement émis par l'insecte lui permettant d'une part d'évaluer son environnement social lorsque cette émission est faible et continue, et d'autre part de signaler un danger par de fortes émissions ponctuelles, le composé prenant alors le rôle de phéromone d'alarme (Francis *et al.* 2005; Verheggen *et al.* 2008b; Verheggen *et al.* 2009b). La capacité des fourmis à détecter des doses d'EBF correspondant aux émissions d'une colonie de pucerons non stressés et à s'en aider dans l'orientation de leurs recherches de partenaires a été mise en évidence ②.

Cependant, étant émis par bon nombre de pucerons tant myrmécophiles que non myrmécophiles (Francis *et al.* 2005), ce seul composé ne permettrait pas leur discrimination à distance par les fourmis, phénomène pourtant observé au cours de ce travail. De plus, malgré la suppression récente d'une colonie de pucerons, et donc de la source d'EBF et autres terpènes éventuellement associés, une plante précédemment infestée reste significativement attractive pour les fourmis exploratrices, corroborant l'existence de paramètres attractifs supplémentaires.

L'origine de cette importante attraction résiduelle est donc à rechercher dans les composés volatils induits par la présence de pucerons, mais non produits par ce dernier. Deux sources potentielles de volatils sont donc à considérer : la plante hôte, capable de modifier ses émissions en réponse à l'attaque des aphidés, ainsi que le miellat accumulé aux abords de la colonie. En utilisant une approche par élimination, aucun effet significatif lié aux volatils potentiels émis par la plante hôte n'a été observé ⑤, tandis que le rôle significatif des composés volatils du miellat a pu être mis en évidence ③. En effet, les fourmis se sont montrées capables de percevoir ces composés et de s'en aider non seulement dans l'orientation de leurs recherches, mais également dans la discrimination à distance de différentes espèces de pucerons. Cette reconnaissance donne lieu à une hiérarchisation de leur choix d'orientation en fonction des espèces disponibles, un puceron non myrmécophile, constituant une proie potentielle, perdant son attractivité en présence d'un puceron myrmécophile, potentielle source de nourriture plus stable. De plus, outre l'information apportée quant à la présence de puceron et l'espèce considérée, les émissions volatiles du miellat sont susceptibles de renseigner le partenaire fourmi sur l'état général de la colonie de pucerons et sur sa productivité de par la quantité de volatils émis ainsi que leur nature, les émissions du miellat se modifiant fortement à la fois qualitativement et quantitativement au cours de son vieillissement. Il est probable que l'information véhiculée par le miellat ainsi que son attractivité soient davantage liées à des ratios entre composés volatils qu'à certains composés en tant que tels, les miellats d'*A. pisum* et *A. fabae* étudiés s'étant montrés qualitativement extrêmement proches.

La production de ces sémiochimiques est assurée par la microflore du miellat, celle-ci produisant une part significative des volatils de cette sécrétion par fermentation et dégradation de ses constituants (Leroy *et al.* 2009). En effet, les modifications directes réalisées par la microflore, et spécialement par le genre *Staphylococcus*, sur divers sucres et acides aminés présents dans le miellat peuvent être à l'origine d'une large gamme de molécules, dont de nombreux composés à 4 et 5 carbones, aux fonctions chimiques diverses, allant d'aldéhydes aux alcools, acides et esters correspondants (Thibout *et al.*

1993; Schulz and Dickschat 2007; Leroy *et al.* 2011a). Ceci expliquerait l'origine de la majorité des composés volatils observés dans les émissions de miellats au cours de ce travail.

Isolé de la microflore d'*Aphis fabae*, *Staphiloccocus xylosus* produit, lorsqu'il est mis en culture dans un milieu adapté précédemment décrit (Leroy *et al.* 2011a), un profil en volatils qualitativement proche de celui du miellat de ce puceron. Ce profil s'est révélé attractif pour les fourmis, contrairement à ceux émis par les autres souches isolées et évaluées au cours de ce travail (mais non identifiées vu leur absence d'effet attractif). Il s'agit là de la première mise en évidence de l'influence que la microflore peut exercer sur cette relation mutualiste.

Ces composés organiques volatils émis par la microflore constituent un signal sur lequel le puceron a peu de prise, réduisant les risques de mimétisme ou de camouflage, et assurant donc ainsi une certaine « honnêteté » du signal.

Enfin, la question de savoir si un puceron recherche lui aussi activement son partenaire mutualiste a été abordée ④, différents auteurs signalant en effet une abondance accrue de pucerons à proximité de fourmilières mais ne sachant conclure si ce phénomène est lié à un choix délibéré de l'insecte ou à sa meilleure survie en ces conditions (Hopkins and Thacker 1999; Bishop and Bristow 2003; Stewart-Jones *et al.* 2008; Minarro *et al.* 2010; Novgorodova and Gavrilyuk 2012). Tant en tunnel de vol qu'en olfactomètre, les pucerons ailés testés n'ont montré aucune attraction significative vers leur partenaire, ni vers une plante hôte fréquentée par ces derniers ④⑤. Par contre, une fois sur la plante hôte, les pucerons ailés y sont significativement retenus par la présence de fourmis. Cette observation corrobore le constat fait par plusieurs auteurs quant à la réduction de la mobilité des pucerons entretenus, par le biais de modifications comportementales ou physiques (Way 1963; Kleinjan and Mittler 1975; Buckley 1987; Hölldobler and Wilson 1990; Guénard *et al.* 2007; Yoo and Holway 2011; Yao 2012; Yao 2014), et l'étend aux premiers contacts entre ces insectes.

Ces données nouvelles ouvrent la voie à une nouvelle approche de la relation mutualiste fourmis-pucerons incluant le rôle des sémiochimiques volatils mais également de la microflore au sein de cette interaction. Sous cet éclairage nouveau, de nombreuses questions se posent en effet. D'une part, une étude approfondie de la microflore du miellat serait à entreprendre dans l'optique de caractériser la variabilité de cette microflore au sein des populations naturelles du puceron modèle étudié, *Aphis fabae*, et d'étudier l'impact d'éventuelles variations sur la composante volatile du miellat et sur l'interaction avec les fourmis, en tenant compte de la variabilité potentielle liée aux facteurs géographiques et temporels. Cette étude serait par la suite à étendre à diverses espèces de pucerons, tant mutualistes que non mutualistes, afin d'investiguer l'existence de souches microbiennes caractéristiques de ces groupes et de mettre en évidence des patterns chimiques responsables de l'influence observée sur le comportement des fourmis. Outre leur effet à présent avéré sur l'attraction des fourmis vers des colonies encore non entretenues, la microflore ainsi que les sémiochimiques qu'elle produit pourraient également avoir un impact sur les relations déjà établies, point restant à étudier. Ces éléments, couplés aux sémiochimiques directement émis par le puceron, pourraient en effet renseigner à distance la fourmi sur divers paramètres liés à l'état général des colonies entretenues, tels que leur niveau de stress, l'évolution de la population et le niveau d'exploitation de la ressource miellat.

Aux côtés des composés non volatils du miellat et paramètres morphologiques, physiologiques et comportementaux du puceron, dont les rôles importants sont déjà bien connus, la composante volatile pourrait donc prendre part intégrante à l'équilibre de la relation mutualiste, et ce depuis l'attraction et la mise en contact des futurs partenaires.

La question du rôle de composés volatils inorganiques, tel le CO_2 par exemple, dans l'attraction des fourmis pourrait également être soulevée. Cependant, ce type de composé étant très peu spécifique, il est probable que son effet, s'il existe, soit plus un effet de synergie avec d'autres volatils plus spécifiques au puceron qu'un effet du composé seul. Ceci reste au stade d'une hypothèse.

Il serait également intéressant de se pencher sur le cas de pucerons myrmécophiles obligatoires. Les enjeux du mutualisme pour ce type de pucerons étant différents, il serait possible que tant leurs communications avec leur partenaire mutualiste que leur capacité à le trouver s'en voient également affectées.

Enfin, l'occurrence des phénomènes observés au cours des recherches effectuées en conditions de laboratoire serait à confirmer en conditions naturelles. La transposition de cette étude aux contraintes multiples du terrain, de natures environnementales, climatiques, biologiques ou techniques, s'avère délicate à mettre en œuvre. En effet, les facteurs influant sur l'état général et les besoins des deux partenaires mutualistes ainsi que sur leur interaction sont multiples et pratiquement impossibles à dissocier et à contrôler efficacement lors d'essais en conditions naturelles. La modification du comportement de la fourmi vis-à-vis d'une espèce de puceron, suite à l'ajout d'une seconde espèce dans le système étudié, observée au cours de cette étude donne un aperçu de l'influence considérable que ces interactions croisées peuvent prendre. En effet, en conditions naturelles, les deux partenaires :

- se retrouvent dans un système ouvert, en situation à la fois de compétition pour un partenariat mutualiste et de choix entre divers partenaires, potentiellement de différentes espèces,
- sont soumis à l'influence de l'alimentation disponible ; tant pour le puceron, dont le type et la qualité de la plante hôte influencent l'état physiologique général et la production de miellat, que pour la fourmi, sur laquelle la disponibilité de proies et de sources de sucres alternatives, telles les nectaries extraflorales, aura un impact comportemental,
- subissent une pression de prédation ainsi que les aléas climatiques, facteurs pouvant être critiques pour leur survie mais également connus pour potentiellement impacter positivement la relation mutualiste,
- sont soumis aux cycles saisonniers, synonymes d'adaptations physiologiques et comportementales à l'approche de l'hiver, telles qu'une période d'hivernation pour la fourmi ou qu'une phase de reproduction sexuée donnant lieux à des œufs capables de survivre à la mauvaise saison dans le cas du puceron. Ceci engendre une cyclicité et une discontinuité dans la relation mutualiste.

Autant de paramètres dont les effets tant isolés que combinés seraient à prendre en compte dans une étude de terrain.

Néanmoins, ces données apporteraient un complément d'information utile, et pourraient permettre la mise en évidence d'interactions avec les multiples composantes de l'écosystème au sein duquel la relation prendra place. D'un point de vue pratique, une première approche serait de ramener ce système complexe à une situation semi-naturelle simplifiée, telles que de grandes cages de vol en tulle englobant la parcelle d'étude, où un certain contrôle des populations d'insectes serait possible. Dans un tel système, une attention particulière serait à porter aux vents, desquels seront tributaires la propagation et la dispersion des composés volatils étudiés. La suppression des sources de volatils potentiellement interférents situées à proximité serait à prévoir dans un rayon suffisant, ainsi qu'un espacement des cages d'essai limitant les risques d'effets croisés.

En termes de perspectives, l'impact des changements climatiques sur les deux partenaires mutualistes et sur la relation qu'ils entretiennent serait également à investiguer. En effet, il semble aujourd'hui évident que la température ainsi que la concentration atmosphérique en gaz à effet de serre augmentent à l'échelle mondiale, et devraient continuer dans cette voie pour les décennies à venir. Ces modifications pourraient influencer le comportement des partenaires mutualistes, directement, ou indirectement par exemple suite à un impact sur la plante hôte. Des travaux en ce sens sont déjà en cours et tendent à démontrer l'impact potentiel de tels changements sur les partenaires mutualistes. En effet, l'augmentation de la teneur en CO_2 atmosphérique influencerait significativement l'émission d'EBF par les pucerons, de même que leur réponse comportementale à ce composé (Boullis *et al.*, 2016). Cette première piste révélant une modification comportementale significative dans l'émission et la réponse à un sémiochimique chez l'un des partenaires serait à poursuivre et à compléter par la caractérisation de l'effet de cette modification sur la relation mutualiste d'une part ; et d'autre part par la recherche d'autres modifications relatives à l'émission de sémiochimiques et aux réponses qu'ils engendrent, tant au niveau des partenaires mutualistes eux-mêmes qu'au niveau des multiples éléments annexes contribuant à cette relation tels la plante hôte ou le miellat et sa microflore.

En final, l'attraction entre fourmis et pucerons est un phénomène que les travaux de laboratoire ont permis de mieux comprendre dans sa complexité. Le rôle de recherche active du puceron semble très limité, ce qui n'est pas le cas de son partenaire mutualiste. Plusieurs vecteurs d'information ont été ciblés ; que ce soit la phéromone d'alarme produite par le puceron ou des composés organiques volatils issus du miellat et de sa métabolisation par des souches bactériennes. Ces données nouvelles complètent un vide dans un modèle biologique déjà largement documenté sur d'autres aspects et ouvrent la voie à de nouvelles pistes de réflexion sur ce modèle mutualiste, mais également sur la place de la microflore et des composés volatils dans les interactions interspécifiques en général.

Références

Bishop DB, Bristow CM (2003) Effects of the presence of the Allegheny mound ant (Hymenoptera : Formicidae) in providing enemy-free space to myrmecophilous aphid and soft scale populations. Ann Entomol Soc Am 96:202–210.

Boullis A, Heuskin S, Lognay GC, et al (2016) Climate change: atmospheric CO2 concentration affect aphid alarm signaling. In: 32nd annual meeting of the ISCE. Iguassu Falls, Brasil

Buckley R (1987) Ant-Plant-Homopteran Interactions. In: Macfadyen A, Ford ED (eds) Advances in Ecological Research. Academic Press, pp 53–85

Francis F, Vandermoten S, Verheggen F, et al (2005) Is the (E)-β-farnesene only volatile terpenoid in aphids? J Appl Entomol 129:6–11.

Guénard B, Lucas É, Francoeur A (2007) Mutualisme fourmis pucerons et guilde aphidiphage associée : le cas de la prédation furtive. Université du Québec à Montréal

Hölldobler B, Wilson EO (1990) The Ants. Springer-Verlag GmbH

Hopkins GW, Thacker JI (1999) Ants and habitat specificity in aphids. J Insect Conserv 3:25–31.

Joachim C, Vosteen I, Weisser WW (2015) The aphid alarm pheromone (E)-β-farnesene does not act as a cue for predators searching on a plant. Chemoecology 25:105–113. doi: 10.1007/s00049-014-0176-z

Joachim C, Weisser WW (2015) Does the Aphid Alarm Pheromone (E)-β-farnesene Act as a Kairomone under Field Conditions? J Chem Ecol 41:267–275. doi: 10.1007/s10886-015-0555-0

Kleinjan JE, Mittler TE (1975) A chemical influence of ants on wing development in aphids. Entomol Exp Appl 18:384–388. doi: 10.1111/j.1570-7458.1975.tb00411.x

Leroy P, Capella Q, Haubruge E (2009) Aphid honeydew impact on the tritrophic relationships between host-plants, phytophagous insects and their natural enemies. Biotechnol Agron Soc Environ 13:325–334.

Leroy PD, Sabri A, Heuskin S, et al (2011) Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. Nat Commun 2:7. doi: Artn 348 Doi 10.1038/Ncomms1347

Minarro M, Fernandez-Mata G, Medina P (2010) Role of ants in structuring the aphid community on apple. Ecol Entomol 35:206–215. doi: DOI 10.1111/j.1365-2311.2010.01173.x

Novgorodova TA, Gavrilyuk A V (2012) The degree of protection different ants (Hymenoptera: Formicidae) provide aphids (Hemiptera: Aphididae) against aphidophages. Eur J Entomol 109:187–196.

Schulz S, Dickschat JS (2007) Bacterial volatiles: The smell of small organisms. Nat Prod Rep 24:814–842. doi: 10.1039/b507392h

Stewart-Jones A, Pope TW, Fitzgerald JD, Poppy GM (2008) The effect of ant attendance on the success of rosy apple aphid populations, natural enemy abundance and apple damage in orchards. Agric For Entomol 10:37–43. doi: DOI 10.1111/j.1461-9563.2007.00353.x

Thibout E, Guillot JF, Auger J (1993) Microorganisms are involved in the production of volatile kairomones affecting the host seeking behaviour of Diadromus pulchellus, a parasitoid of Acrolepiopsis assectella. Physiol Entomol 18:176–182.

Verheggen FJ, Haubruge E, Moraes CM de, Mescher MC (2009) Social environment influences aphid production of alarm pheromone. Behav Ecol 20:283–288. doi: 10.1093/beheco/arp009

Verheggen FJ, Mescher MC, Haubruge E, et al (2008) Emission of alarm pheromone in aphids: a non-contagious phenomenon. J Chem Ecol 34:1146–1148. doi: 10.1007/s10886-008-9528-x

Vosteen I, Weisser WW, Kunert G (2016) Is there any evidence that aphid alarm pheromones work as prey and host finding kairomones for natural enemies? Ecol Entomol 41:1–12. doi: 10.1111/een.12271

Way MJ (1963) Mutualism between ants and honeydew-producing Homoptera. Annu Rev Entomol 8:307–344.

Yao I (2012) Ant attendance reduces flight muscle and wing size in the aphid Tuberculatus quercicola. Biol Lett 8:624–627. doi: 10.1098/rsbl.2012.0014 10.1034/j.1600-0706.2000.890101.x)

Yao I (2014) Costs and constraints in aphid-ant mutualism. Ecol Res 29:383–391. doi: 10.1007/s11284-014-1151-4

Yoo HJS, Holway DA (2011) Context-dependence in an ant-aphid mutualism: direct effects of tending intensity on aphid performance. Ecol Entomol 36:450–458. doi: DOI 10.1111/j.1365-2311.2011.01288.x

CHAPITRE VIII

RÉFÉRENCES BIBLIOGRAPHIQUES

Abassi SAL, Birkett MA, Pettersson J, et al (2000) Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. J Chem Ecol 26:1765–1771. doi: 10.1023/A:1005555300476

Almohamad R, Verheggen FJ, Francis F, et al (2008) Emission of alarm pheromone by non-preyed aphid colonies. J Appl Entomol 132:601–604. doi: 10.1111/j.1439-0418.2008.01309.x

Altschul SF, Madden TL, Schäffer AA, et al (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. doi: 10.1093/nar/25.17.3389

Álvarez-Pérez S, Herrera CM, de Vega C (2012) Zooming-in on floral nectar: A first exploration of nectar-associated bacteria in wild plant communities. FEMS Microbiol Ecol 80:591–602. doi: 10.1111/j.1574-6941.2012.01329.x 10.1111/j.1365-294X.2011.05402.x

Attygalle AB, Evershed RP, Morgan ED, Cammaerts MC (1983) Dufour gland secretions of workers of the ants Myrmica sulcinodis and Myrmica lobicornis, and comparison with six other species of Myrmica. Insect Biochem 13:507–512.

Attygalle AB, Morgan ED (1985) Ant trail pheromones. Adv In Insect Phys 18:1–30.

Attygalle AB, Morgan ED (1984) Identification of trail pheromone of the ant Tetramorium caespitum L. (Hymenoptera: Myrmicinae). J Chem Ecol 10:1453–1468.

Beattie AJ (1985) The Evolutionary Ecology of Ant–Plant Mutualisms. publisherNameCambridge University Press

Beck HC, Hansen AM, Lauritsen FR (2004) Catabolism of leucine to branched-chain fatty acids in Staphylococcus xylosus. J Appl Microbiol 96:1185–1193. doi: 10.1111/j.1365-2672.2004.02253.x

Billen J (2009) Diversity and Morphology of Exocrine Glands in Ants. In: XIX Simposio de Mirmecologia. Ouro Preto (Brasil), pp 1–6

Bishop DB, Bristow CM (2003) Effects of the presence of the Allegheny mound ant (Hymenoptera : Formicidae) in providing enemy-free space to myrmecophilous aphid and soft scale populations. Ann Entomol Soc Am 96:202–210.

Blaiotta G, Pennacchia C, Parente E, Villani F (2003) Design and Evaluation of Specific PCR Primers for Rapid and Reliable Identification of Staphylococcus xylosus Strains Isolated from Dry Fermented Sausages. Syst Appl Microbiol 26:601–610. doi: http://dx.doi.org/10.1078/072320203770865918

Boucher DH, James S, Keeler KH (1982) The ecology of mutualism. Annu Rev Ecol Syst Vol 13 315–347.

Boullis A, Heuskin S, Lognay GC, et al (2016) Climate change: atmospheric CO2 concentration affect aphid alarm signaling. In: 32nd annual meeting of the ISCE. Iguassu Falls, Brasil.

Breton LM, Addicott JF (1992) Density-Dependent Mutualism in an Aphid-Ant Interaction. Ecology 73:2175–2180.

Bristow CM (1991) Why are so few aphids ant-tended? In: Cutler DF, Huxley C (eds) Ant/Plant Interactions. Oxford University Press, pp 104–119

Bronstein JL, Alarcon R, Geber M (2006) The evolution of plant-insect mutualisms. New Phytol 172:412–428. doi: 10.1111/j.1469-8137.2006.01864.x

Bronstein JL, Dieckmann U, Ferrière R (2004) Coevolutionary Dynamics and the Conservation of Mutualisms. Evol Conserv Biol 305–326.

Brysch-Herzberg M (2004) Ecology of yeasts in plant-bumblebee mutualism in Central Europe. FEMS Microbiol Ecol 50:87–100. doi: 10.1016/j.femsec.2004.06.003

Buckley R (1987) Ant-Plant-Homopteran Interactions. In: Macfadyen A, Ford ED (eds) Advances in Ecological Research. Academic Press, pp 53–85

Buffin A, Mailleux AC, Detrain C, Deneubourg JL (2011) Trophallaxis in Lasius niger: A variable frequency and constant duration for three food types. Insectes Soc 58:177–183. doi: 10.1007/s00040-010-0133-y

Byers JA, Fefer D, Levi-Zada A (2013) Sex pheromone component ratios and mating isolation among three Lygus plant bug species of North America. Naturwissenschaften 100:1115–1123. doi: 10.1007/s00114-013-1113-7

Cammaerts MC, Evershed RP, Morgan ED (1981) Comparative study of the mandibular gland secretion of four species of Myrmica ants. J Insect Physiol 27:225–231.

Cardé RT, Millar JG (2009) Chapter 195 - Pheromones. In: Cardé VHRTBT-E of I (Second E (ed) Encyclopedia of Insects. Academic Press, San Diego, pp 766–772

Castracani C, Tamarri V, Grasso DA, et al (2008) Chemical communication in mating behaviour of the slave-making ant Polyergus rufescens (Hymenoptera, Formicidae): 3-Ethyl-4-methylpentanol as a critical component of the queen sex pheromone. Insectes Soc 55:137–143. doi: 10.1007/s00040-008-0981-x

Censier F, Fischer CY, Chavalle S, et al (2014) Identification of 1-methyloctyl butanoate as the major sex pheromone component from females of the saddle gall midge, Haplodiplosis marginata (Diptera: Cecidomyiidae). Chemoecology 24:243–251. doi: 10.1007/s00049-014-0167-0

Davidson EW, Rosell RC, Hendrix DL (2000) Culturable bacteria associated with the whitefly, Bemisia Argentifolii (Homoptera: Aleyrodidae). Florida Entomol 83:159–171.

Detrain C, Verheggen FJ, Diez L, et al (2010) Aphid-ant mutualism: how honeydew sugars influence the behaviour of ant scouts. Physiol Entomol 35:168–174.

Devigne C, Detrain C (2002) Collective exploration and area marking in the ant Lasius niger. Insectes Soc 49:357–362.

Devigne C, Detrain C (2006) How does food distance influence foraging in the ant Lasius niger: The importance of home-range marking. Insectes Soc 53:46–55. doi: 10.1007/s00040-005-0834-9

Dixon AFG (1985) Aphid ecology. Blackie ; Distributed in the U.S.A. by Chapman and Hall, Glasgow

Dixon AFG, Howard MT (1986) Dispersal in Aphids, A Problem in Resource Allocation. In: Danthanarayana W (ed) Insect Flight. Springer Berlin Heidelberg, pp 145–151

Douglas AE (1998) Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria Buchnera. Annu Rev Entomol 43:17–37. doi: 10.1146/annurev.ento.43.1.17

Durieux D, Verheggen FJ, Vandereycken A, et al (2010) Review: chemical ecology of ladybird beetles. Biotechnol Agron Soc Environ 14:351–367.

Edwards LJ, Siddall JB, Dunham LL, et al (1973) Trans-β-farnesene, alarm pheromone of the green peach aphid, Myzus persicae (Sulzer) [15]. Nature 241:126–127. doi: 10.1038/241126b0

El-Ziady S, Kennedy JS (1956) Beneficial effects of the common garden ant, Lasius niger L., on the black bean aphid, Aphis fabae Scopoli. Proc R Ent Soc Lond 31:5.

Endo S, Itino T (2012) Myrmecophilous aphids produce cuticular hydrocarbons that resemble those of their tending ants. Popul Ecol 1–8. doi: 10.1007/s10144-012-0355-0

Fassotte B, Fischer C, Durieux D, et al (2014) First evidence of a volatile sex pheromone in lady beetles. PLoS One. doi: 10.1371/journal.pone.0115011

Febvay G, Rahbe Y, Rynkiewicz M, et al (1999) Fate of dietary sucrose and neosynthesis of amino acids in the pea aphid, acyrthosiphon pisum, reared on different diets. J Exp Biol 202 (Pt 19:2639–2652.

Fischer CY, Laurent P, Heuskin S, et al (2012) Fast-GC quantification of harmonine, the major defense alkaloid of the multicolored Asian ladybird Harmonia axyridis (Pallas). Am J Anal Chem 3:67–75.

Fischer CY, Lognay GC (2012) Simple and Automatic Closed Grinding and Extraction System. J Chem Educ 89:1611–1612. doi: 10.1021/ed2007907

Fischer CY, Lognay GC, Detrain C, et al (2015a) Bacteria may enhance species association in an ant–aphid mutualistic relationship. Chemoecology 25:223–232. doi: 10.1007/s00049-015-0188-3

Fischer CY, Vanderplanck M, Lognay GC, et al (2015b) Do aphids actively search for ant partners? Insect Sci 22:283–288. doi: 10.1111/1744-7917.12125

Fischer MK, Hoffmann KH, Völkl W (2001) Competition for mutualists in an ant-homopteran interaction mediated by hierarchies of ant attendance. Oikos 92:531–541.

Fischer MK, Shingleton AW (2001) Host plant and ants influence the honeydew sugar composition of aphids. Funct Ecol 15:544–550.

Fischer MK, Volkl W, Hoffmann KH (2005) Honeydew production and honeydew sugar composition of polyphagous black bean aphid, Aphis fabae (Hemiptera: Aphididae) on various host plants and implications for ant-attendance. Eur J Entomol 102:155–160.

Fischer MK, Volkl W, Schopf R, Hoffmann KH (2002) Age-specific patterns in honeydew production and honeydew composition in the aphid Metopeurum fuscoviride: implications for ant-attendance. J Insect Physiol 48:319–326.

Francis F, Vandermoten S, Verheggen F, et al (2005) Is the (E)-β-farnesene only volatile terpenoid in aphids? J Appl Entomol 129:6–11.

Glinwood R, Willekens J, Pettersson J (2003) Discrimination of aphid mutualists by an ant based on chemical cues. Acta Agric Scand Sect B-Soil Plant Sci 53:177–182. doi: Doi 10.1080/09064710310015445

Goggin FL (2007) Plant-aphid interactions: molecular and ecological perspectives. Curr Opin Plant Biol 10:399–408. doi: 10.1016/j.pbi.2007.06.004

Goldansaz SH, McNeil JN (2006) Effect of wind speed on the pheromone-mediated behavior of sexual morphs of the potato aphid, Macrosiphum euphorbiae (Thomas) under laboratory and field conditions. J Chem Ecol 32:1719–1729.

González-Teuber M, Eilmus S, Muck A, et al (2009) Pathogenesis-related proteins protect extrafloral nectar from microbial infestation. Plant J 58:464–473.

González-Teuber M, Heil M (2010) Pseudomyrmex ants and acacia host plants join efforts to protect their mutualism from microbial threats. Plant Signal Behav 5:890–892.

Grenier AM, Nardon C, Rahbé Y (1994) Observations on the micro-organisms occurring in the gut of the pea aphid Acyrthosiphon pisum. Entomol Exp Appl 70:91–96. doi: 10.1111/j.1570-7458.1994.tb01762.x

Guénard B, Lucas É, Francoeur A (2007) Mutualisme fourmis pucerons et guilde aphidiphage associée : le cas de la prédation furtive. Université du Québec à Montréal

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98.

Han B-Y, Wang M-X, Zheng Y-C, et al (2014) Sex pheromone of the tea aphid, Toxoptera aurantii (Boyer de Fonscolombe) (Hemiptera: Aphididae). Chemoecology 24:179–187. doi: 10.1007/s00049-014-0161-6

Harada H, Ishikawa H (1997) Experimental pathogenicity of Erwinia aphidicola to pea aphid, Acyrthosiphon pisum. J Gen Appl Microbiol 43:363–367.

Haynes S, Darby AC, Daniell TJ, et al (2003) Diversity of Bacteria Associated with Natural Aphid Populations. Appl Environ Microbiol 69:7216–7223. doi: 10.1128/aem.69.12.7216-7223.2003

Herbert JJ, Horn DJ (2008) Effect of Ant Attendance by Monomorium minimum (Buckley) (Hymenoptera: Formicidae) on Predation and Parasitism of the Soybean Aphid Aphis glycines Matsumura (Hemiptera: Aphididae). Environ Entomol 37:1258–1263.

Herre EA, Knowlton N, Mueller UG, Rehner SA (2016) The evolution of mutualisms: exploring the paths between conflict and cooperation. Trends Ecol Evol 14:49–53. doi: 10.1016/S0169-5347(98)01529-8

Herrera CM, Canto A, Pozo MI, Bazaga P (2010) Inhospitable sweetness: Nectar filtering of pollinator-borne inocula leads to impoverished, phylogenetically clustered yeast communities. Proc R Soc B Biol Sci 277:747–754. doi: 10.1098/rspb.2009.1485 10.1034/j.1600-0706.2000.910301.x

Herrera CM, de Vega C, Canto A, Pozo MI (2009) Yeasts in floral nectar: a quantitative survey. Ann Bot 103:1415–1423. doi: 10.1093/aob/mcp026

Herrera CM, García IM, Pérez R (2008) Invisible floral larcenies: Microbial communities degrade floral nectar of bumble bee-pollinated plants. Ecology 89:2369–2376. doi: 10.1890/08-0241.1

Hölldobler B, Plowes NJR, Johnson RA, et al (2013) Pygidial gland chemistry and potential alarm-recruitment function in column foraging, but not solitary, Nearctic Messor harvesting ants (Hymenoptera: Formicidae: Myrmicinae). J Insect Physiol 59:863–869.

Hölldobler B, Wilson EO (1990) The Ants. Springer-Verlag GmbH

Holman J (2009) The Aphids and their Host Plants. In: Host Plant Catalog of Aphids. Springer Netherlands, pp 7–651

Hopkins GW, Thacker JI (1999) Ants and habitat specificity in aphids. J Insect Conserv 3:25–31.

Hussain A, Forrest JM, Dixon AF (1974) Sugar, organic acid, phenolic acid and plant growth regulator content of extracts of honeydew of the aphid Myzus persicae and of its host plant, Raphanus sativus. Ann Appl Biol 78:65–73. doi: 10.1111/j.1744-7348.1974.tb01486.x

Joachim C, Vosteen I, Weisser WW (2015) The aphid alarm pheromone (E)-β-farnesene does not act as a cue for predators searching on a plant. Chemoecology 25:105–113. doi: 10.1007/s00049-014-0176-z

Joachim C, Weisser WW (2015) Does the Aphid Alarm Pheromone (E)-β-farnesene Act as a Kairomone under Field Conditions? J Chem Ecol 41:267–275. doi: 10.1007/s10886-015-0555-0

Johnson B (1959) Ants and form reversal in aphids. Nature 184:740–741. doi: 10.1038/184740a0

Katayama N, Suzuki N (2003) Bodyguard effects for aphids of Aphis craccivora Koch (Homoptera : Aphididae) as related to the activity of two ant species, Tetramorium caespitum Linnaeus (Hymenoptera : Formicidae) and Lasius niger L. (Hymenoptera : Formicidae). Appl Entomol Zool 38:427–433.

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: The conservation of plant-pollinator interactions. Annu Rev Ecol Syst 29:83–112. doi: 10.1146/annurev.ecolsys.29.1.83

Kirzinger MWB, Stavrinides J (2012) Host specificity determinants as a genetic continuum. Trends Microbiol 20:88–93. doi: http://dx.doi.org/10.1016/j.tim.2011.11.006

Kiss A (1981) Melezitose, aphids and ants. Oikos 37:382.

Kleinjan JE, Mittler TE (1975) A chemical influence of ants on wing development in aphids. Entomol Exp Appl 18:384–388. doi: 10.1111/j.1570-7458.1975.tb00411.x

Kloos WE (1980) Natural populations of the genus Staphylococcus. Annu Rev Microbiol 34:559–592.

Kost C, Heil M (2006) Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. J Ecol 94:619–628.

Kremen C, Williams NM, Aizen M a., et al (2007) Pollination and other ecosystem services produced by mobile organisms: A conceptual framework for the effects of land-use change. Ecol Lett 10:299–314. doi: 10.1111/j.1461-0248.2007.01018.x

Kunert G, Otto S, Röse USR, et al (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. Ecol Lett 8:596–603.

Kunert G, Reinhold C, Gershenzon J (2010) Constitutive emission of the aphid alarm pheromone, (E)-beta-farnesene, from plants does not serve as a direct defense against aphids. BMC Ecol 10:(23 November 2010)–(23 November 2010).

Lang C, Menzel F (2011) Lasius niger ants discriminate aphids based on their cuticular hydrocarbons. Anim Behav 82:1245–1254. doi: http://dx.doi.org/10.1016/j.anbehav.2011.08.020

Leroy P, Capella Q, Haubruge E (2009) Aphid honeydew impact on the tritrophic relationships between host-plants, phytophagous insects and their natural enemies. Biotechnol Agron Soc Environ 13:325–334.

Leroy PD, Sabri A, Heuskin S, et al (2011a) Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. Nat Commun 2:7. doi: Artn 348Doi 10.1038/Ncomms1347

Leroy PD, Wathelet B, Sabri A, et al (2011b) Aphid-host plant interactions: does aphid honeydew exactly reflect the host plant amino acid composition? Arthropod Plant Interact 5:193–199. doi: DOI 10.1007/s11829-011-9128-5

Liepert C, Dettner K (1996) Role of cuticular hydrocarbons of aphid parasitoids in their relationship to aphid-attending ants. J Chem Ecol 22:695–707.

Liu Y, Liu Y (2002) Identification of recruitment pheromones in the harvester ant genus Pogonomyrmex. Fenxi Huaxue 30:298–300.

Lohman DJ, Liao Q, Pierce NE (2006) Convergence of chemical mimicry in a guild of aphid predators. Ecol Entomol 31:41–51. doi: 10.1111/j.0307-6946.2006.00758.x

Mailleux AC, Buffin A, Detrain C, Deneubourg JL (2011) Recruitment in starved nests: The role of direct and indirect interactions between scouts and nestmates in the ant Lasius niger. Insectes Soc 58:559–567. doi: 10.1007/s00040-011-0177-7

Mailleux AC, Deneubourg JL, Detrain C (2003a) Regulation of ants' foraging to resource productivity. Proc R Soc London Ser B-Biological Sci 270:1609–1616. doi: DOI 10.1098/rspb.2003.2398

Mailleux AC, Deneubourg JL, Detrain C (2000) How do ants assess food volume? Anim Behav 59:1061–1069.

Mailleux AC, Deneubourg JL, Detrain C (2003b) How does colony growth influence communication in ants? Insectes Soc 50:24–31. doi: 10.1007/s000400300004

Mailleux AC, Detrain C, Deneubourg JL (2006) Starvation drives a threshold triggering communication. J Exp Biol 209:4224–4229. doi: 10.1242/jeb.02461

Mailleux AC, Detrain C, Deneubourg JL (2005) Triggering and persistence of trail-laying in foragers of the ant Lasius niger. J Insect Physiol 51:297–304. doi: 10.1016/j.jinsphys.2004.12.001

Mailleux AC, Devigne C, Deneubourg JL, Detrain C (2010) Impact of starvation on lasius niger' exploration. Ethology 116:248–256. doi: 10.1111/j.1439-0310.2009.01736.x

Martineau F, Picard FJ, Ménard C, et al (2000) Development of a rapid PCR assay specific for Staphylococcus saprophyticus and application to direct detection from urine samples. J Clin Microbiol 38:3280–3284.

Micha SG, Wyss U (1996) Aphid alarm pheromone (E)-β-farnesene: a host finding kairomone for the aphid primary parasitoid Aphidius uzbekistanicus (Hymenoptera: Aphidiinae). Chemoecology 7:132–139.

Minarro M, Fernandez-Mata G, Medina P (2010) Role of ants in structuring the aphid community on apple. Ecol Entomol 35:206–215. doi: DOI 10.1111/j.1365-2311.2010.01173.x

Mittler TE (1958) The feeding and nutrition of large willow aphid (Tuberolachnus salignus). II. The nitrogen and sugar composition of ingested phloem sap and excreted honeydew. J Exp Biol 35:74–84.

Morgan ED (2009) Trail pheromones of ants. Physiol Entomol 34:1–17. doi: 10.1111/j.1365-3032.2008.00658.x

Morgan ED (2008) Chemical sorcery for sociality: Exocrine secretions of ants (Hymenoptera: Formicidae). Myrmecological News 11:79–90.

Morot-Bizot S, Talon R, Leroy-Setrin S (2003) Development of specific PCR primers for a rapid and accurate identification of Staphylococcus xylosus, a species used in food fermentation. J Microbiol Methods 55:279–286. doi: http://dx.doi.org/10.1016/S0167-7012(03)00159-3

Nault LR, Montgomery ME, Bowers WS (1976) Ant-aphid association: role of aphid alarm pheromone. Sci USA 192:1349–1351.

Nielsen C, Agrawal AA, Hajek AE (2010) Ants defend aphids against lethal disease. Biol Lett 6:205–208. doi: DOI 10.1098/rsbl.2009.0743

Novgorodova TA, Gavrilyuk A V (2012) The degree of protection different ants (Hymenoptera: Formicidae) provide aphids (Hemiptera: Aphididae) against aphidophages. Eur J Entomol 109:187–196.

Oliver TH, Leather SR, Cook JM (2009) Tolerance traits and the stability of mutualism. Oikos 118:346–352. doi: DOI 10.1111/j.1600-0706.2008.17045.x

Oliver TH, Leather SR, Cook JM (2012) Ant larval demand reduces aphid colony growth rates in an ant-aphid interaction. Insects 3:120–130. doi: 10.3390/insects3010120

Oliver TH, Mashanova A, Leather SR, et al (2007) Ant semiochemicals limit apterous aphid dispersal. Proc Biol Sci 274:3127–3131. doi: 10.1098/rspb.2007.1251

Outreman Y, Kunert G, Simon J-C, Weisser W (2010) Ecological Costs of Alarm Signalling in Aphids. In: Kindlmann P, Dixon AFG, Michaud JP (eds) Aphid Biodiversity under Environmental Change SE - 10. Springer Netherlands, pp 171–181

Paris CI, Llusia J, Peñuelas J (2010) Changes in monoterpene emission rates of quercus ilex infested by aphids tended by native or invasive lasius ant species. J Chem Ecol 36:689–698. doi: 10.1007/s10886-010-9815-1

Parry K, Morgan ED (1979) Pheromones of ants: a review. Physiol Entomol 4:161–189. doi: 10.1111/j.1365-3032.1979.tb00193.x

Pickett JA, Glinwood RT (2007) Chemical ecology. In: Aphids as Crop Pests. Biological Chemistry Division, Rothamsted Research, Harpenden, Herts, AL5 2JQ, United Kingdom, pp 235–260

Pickett JA, Griffiths DC (1980) Composition of aphid alarm pheromones. J Chem Ecol 6:349–360.

Pickett JA, Wadhams LJ, Woodcock CM, Hardie J (1992) The chemical ecology of aphids. Annu Rev Entomol 67–90.

Plowes NJR, Colella T, Johnson RA, Hölldobler B (2014) Chemical communication during foraging in the harvesting ants Messor pergandei and Messor andrei. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol 200:129–137.

Portha S, Deneubourg JL, Detrain C (2004) How food type and brood influence foraging decisions of Lasius niger scouts. Anim Behav 68:115–122. doi: DOI 10.1016/j.anbehav.2003.10.016

Powell BE, Silverman J (2010) Population Growth of Aphis gossypii and Myzus persicae (Hemiptera: Aphididae) in the Presence of Linepithema humile and Tapinoma sessile (Hymenoptera: Formicidae). Environ Entomol 39:1492–1499. doi: Doi 10.1603/En09211

Renault CK, Buffa LM, Delfino MA (2005) An aphid-ant interaction: effects on different trophic levels. Ecol Res 20:71–74. doi: DOI 10.1007/s11284-004-0015-8

Sakata H (1999) Indirect interactions between two aphid species in relation to ant attendance. Ecol Res 14:329–340.

Sakata H (1995) Density-dependent predation of the ant Lasius niger (Hymenoptera: Formicidae) on two attended aphids Lachnus tropicalis and Myzocallis kuricola (Homoptera: Aphididae). Res Popul Ecol (Kyoto) 37:159–164.

Schulz S, Dickschat JS (2007) Bacterial volatiles: The smell of small organisms. Nat Prod Rep 24:814–842. doi: 10.1039/b507392h

Seibert TF (1992) Mutualistic Interactions of the Aphid Lachnus-Allegheniensis (Homoptera, Aphididae) and Its Tending Ant Formica-Obscuripes (Hymenoptera, Formicidae). Ann Entomol Soc Am 85:173–178.

Shik JZ, Kay AD, Silverman J (2014) Aphid honeydew provides a nutritionally balanced resource for incipient Argentine ant mutualists. Anim Behav 95:33–39. doi: 10.1016/j.anbehav.2014.06.008

Shik JZ, Silverman J (2013) Towards a nutritional ecology of invasive establishment: aphid mutualists provide better fuel for incipient Argentine ant colonies than insect prey. Biol Invasions 15:829–836. doi: 10.1007/s10530-012-0330-x

Shingleton AW, Stern DL, Foster WA (2005) The origin of a mutualism: A morphological trait promoting the evolution of ant-aphid mutualisms. Evolution (N Y) 59:921–926.

Stadler B (1997) The relative importance of host plants, natural enemies and ants in the evolution of life-history characters in aphids. In: Dettner K, Bauer G, Völkl W (eds) Vertical food web interactions : evolutionary patterns and driving forces. Springer -Verlag, pp 241–256

Stadler B, Dixon AFG (2005) Ecology and evolution of aphid-ant interactions. Annu Rev Ecol Evol Syst 36:345–372.

Stadler B, Dixon AFG (1998) Costs of ant attendance for aphids. J Anim Ecol 67:454–459.

Stadler B, Dixon AFG (2008) Mutualism: Ants and their Insect Partners. Department of Animal Ecology, Bayreuth Institute for Terrestrial Ecosystem Research, University of Bayreuth, Germany

Stadler B, Dixon AFG (1999) Ant attendance in aphids: why different degrees of myrmecophily? Ecol Entomol 24:363–369.

Stadler B, Fiedler K, Kawecki TJ, Weisser WW (2001) Costs and benefits for phytophagous myrmecophiles: When ants are not always available. Oikos 92:467–478.

Staudt M, Jackson B, El-Aouni H, et al (2010) Volatile organic compound emissions induced by the aphid Myzus persicae differ among resistant and susceptible peach cultivars and a wild relative. Tree Physiol 30:1320–1334.

Stewart-Jones A, Pope TW, Fitzgerald JD, Poppy GM (2008) The effect of ant attendance on the success of rosy apple aphid populations, natural enemy abundance and apple damage in orchards. Agric For Entomol 10:37–43. doi: DOI 10.1111/j.1461-9563.2007.00353.x

Styrsky JD, Eubanks MD (2007) Ecological consequences of interactions between ants and honeydew-producing insects. Proc R Soc B Biol Sci 274:151–164. doi: 10.1098/rspb.2006.3701 10.1007/BF00325261

Suchet C (2010) Ecologie et Evolution des odeurs florales chez Antirrhinum Majus. Université de Toulouse

Sudd J (1967) An introduction to the behaviour of ants. Edwards Arnold, London

Tegelaar K, Hagman M, Glinwood R, et al (2012) Ant-aphid mutualism: The influence of ants on the aphid summer cycle. Oikos 121:61–66. doi: 10.1111/j.1600-0706.2011.19387.x

Thibout E, Guillot JF, Auger J (1993) Microorganisms are involved in the production of volatile kairomones affecting the host seeking behaviour of Diadromus pulchellus, a parasitoid of Acrolepiopsis assectella. Physiol Entomol 18:176–182.

Turchin P, Kareiva P (1989) Aggregation in Aphis varians: an effective strategy for reducing predation risk. Ecology 70:1008–1016.

Vannette RL, Gauthier MPL, Fukami T (2013) Nectar bacteria, but not yeast, weaken a plant - Pollinator mutualism. Proc R Soc B Biol Sci. doi: 10.1098/rspb.2012.2601

Vantaux A, Parmentier T, Billen J, Wenseleers T (2012) Do Lasius niger ants punish low-quality black bean aphid mutualists? Anim Behav 83:257–262. doi: 10.1016/j.anbehav.2011.10.035

Vantaux A, Schillewaert S, Parmentier T, et al (2015) The cost of ant attendance and melezitose secretion in the black bean aphid Aphis fabae. Ecol. Entomol.

Vantaux A, Van den Ende W, Billen J, Wenseleers T (2011) Large interclone differences in melezitose secretion in the facultatively ant-tended black bean aphid Aphis fabae. J Insect Physiol 57:1614–1621. doi: 10.1016/j.jinsphys.2011.08.014

Ventura M, Elli M, Reniero R, Zink R (2001) Molecular microbial analysis of Bifidobacterium isolates from different environments by the species-specific amplified ribosomal DNA restriction analysis (ARDRA). FEMS Microbiol Ecol 36:113–121. doi: http://dx.doi.org/10.1016/S0168-6496(01)00123-4

Verheggen F (2008) Production of alarm pheromone in aphids and perception by ants and natural enemies. 197.

Verheggen F, Diez L, Detrain C, Haubruge E (2009a) Aphid-ant mutualism: an outdoor study of the benefits for /i Aphis fabae. Biotechnol Agron Soc Environ 13:235–242.

Verheggen FJ, Arnaud L, Bartram S, et al (2008a) Aphid and plant volatiles induce oviposition in an aphidophagous hoverfly. J. Chem. Ecol. 34:301–307.

Verheggen FJ, Diez L, Sablon L, et al (2012) Aphid Alarm Pheromone as a Cue for Ants to Locate Aphid Partners. PLoS One 7:e41841. doi: 10.1371/journal.pone.0041841

Verheggen FJ, Fagel Q, Heuskin S, et al (2007) Electrophysiological and behavioral responses of the multicolored Asian lady beetle, Harmonia axyridis Pallas, to sesquiterpene semiochemicals. J Chem Ecol 33:2148–2155.

Verheggen FJ, Haubruge E, Moraes CM de, Mescher MC (2009b) Social environment influences aphid production of alarm pheromone. Behav Ecol 20:283–288. doi: 10.1093/beheco/arp009

Verheggen FJ, Mescher MC, Haubruge E, et al (2008b) Emission of alarm pheromone in aphids: a non-contagious phenomenon. J Chem Ecol 34:1146–1148. doi: 10.1007/s10886-008-9528-x

Vilcinskas A (2016) Biology and Ecology of Aphids. CRC Press, Taylor & Francis Group

Völkl W, Woodring J, Fischer M, et al (1999) Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. Oecologia 118:483–491.

Vosteen I, Weisser WW, Kunert G (2016) Is there any evidence that aphid alarm pheromones work as prey and host finding kairomones for natural enemies? Ecol Entomol 41:1–12. doi: 10.1111/een.12271

Way MJ (1963) Mutualism between ants and honeydew-producing Homoptera. Annu Rev Entomol 8:307–344.

Wheeler W (1926) Ants, their structure, development and behaviour. Columbia University Press, Lancaster

Wiens F, Zitzmann A, Lachance MA, et al (2008) Chronic intake of fermented floral nectar by wild treeshrews. Proc Natl Acad Sci U S A 105:10426–10431. doi: 10.1073/pnas.0801628105

Wilder SM, Barnum TR, Holway DA, et al (2013) Introduced fire ants can exclude native ants from critical mutualist-provided resources. Oecologia 172:197–205. doi: 10.1007/s00442-012-2477-7

Wilder SM, Holway DA, Suarez A V, Eubanks MD (2011) Macronutrient content of plant-based food affects growth of a carnivorous arthropod. Ecology 92:325–332.

Wilson EO (1955) A Monographic Revision of the Ant Genus Lasius. Bull. Mus. Comp. Zool. 113:59–77.

Wood WF, Hoang TT, McGlynn TP (2011) Volatile components from the mandibular glands of the turtle ants, Cephalotes alfaroi and Cephalotes cristatus. Biochem Syst Ecol 39:135–138.

Wood WF, Palmer TM, Stanton ML (2002) A comparison of volatiles in mandibular glands from three Crematogaster ant symbionts of the whistling thorn acacia. Biochem Syst Ecol 30:217–222.

Woodring J, Wiedemann R, Volkl W, Hoffmann KH (2007) Oligosaccharide synthesis regulates gut osmolality in the ant-attended aphid Metopeurum fuscoviride but not in the unattended aphid Macrosiphoniella tanacetaria. J Appl Entomol 131:1–7. doi: 10.1111/j.1439-0418.2006.01091.x

Yao I (2014) Costs and constraints in aphid-ant mutualism. Ecol Res 29:383–391. doi: 10.1007/s11284-014-1151-4

Yao I (2012) Ant attendance reduces flight muscle and wing size in the aphid Tuberculatus quercicola. Biol Lett 8:624–627. doi: 10.1098/rsbl.2012.0014 10.1034/j.1600-0706.2000.890101.x)

Yao I, Akimoto S (2001) Ant attendance changes the sugar composition of the honeydew of the drepanosiphid aphid Tuberculatus quercicola. Oecologia 128:36–43.

Yao I, Shibao H, Akimato S (2000) Costs and benefits of ant attendance to the drepanosiphid aphid Tuberculatus quercicola. Oikos 89:3–10.

Yoo HJS, Holway DA (2011) Context-dependence in an ant-aphid mutualism: direct effects of tending intensity on aphid performance. Ecol Entomol 36:450–458. doi: DOI 10.1111/j.1365-2311.2011.01288.x

Zhu J, Cossé AA, Obrycki JJ, et al (1999) Olfactory reactions of the twelve-spotted lady beetle, Coleomegilla maculata and the green lacewing, Chrysoperla carnea to semiochemicals released from their prey and host plant: electroantennogram and behavioral responses. J Chem Ecol 25:1163–1177.

CHAPITRE IX

ANNEXES

PUBLICATIONS ET COMMUNICATIONS

Thématiques directement liées à la thèse

Publications

Journaux à comité de lecture

- **Fischer, C.**, Detrain, C., Thonart, P., Haubruge, E., Francis, F., Verheggen, F., & Lognay, G. (2016). Bacteria may contribute to distant species recognition in ant-aphid mutualistic relationships. *Insect Science*, DOI 10.1111/1744-7917.12302

- **Fischer, C.**, Lognay, G., Detrain, C., Piraux, C., Grigorescu, A., Thonart, P., Haubruge, E., Heil, M., & Verheggen, F. (2015), Bacteria may enhance species association in an ant-aphid mutualistic relationship. *Chemoecology*, 1-10, DOI 10.1007/s00049-015-0188-3

- **Fischer, C.**, Vanderplanck, M., Lognay, G., Detrain, C., & Verheggen, F. (2014). Do aphids actively search for ant partners? *Insect Science*, 00, 1–6, DOI 10.1111/1744-7917.12125

- Verheggen, F., Diez, L., Sablon, L., **Fischer, C.**, Bartram, S., Haubruge, E., & Detrain, C. (2012). Aphid Alarm Pheromone as a Cue for Ants to Locate Aphid Partners. *PLoS ONE* 7(8): e41841, DOI:10.1371/journal.pone.0041841

- **Fischer, C**, Lognay, G. (2012). Simple and automatic closed grinding and extraction system. *Journal of Chemical Education.* 89 (12), 1611–1612

Communications

- **Fischer, C.**, O., Lognay, G., Haubruge, E., Detrain, C., & Verheggen, F. (2013, January). Ant-aphid mutualism – How does it start? Presented at BDIV Seminars (Université Catholique de Louvain), Louvain-la-Neuve, Belgium.

- **Fischer, C.**, Piraux, O., Lognay, G., Haubruge, E., Detrain, C., & Verheggen, F. (2012, July). Ant-aphid mutualism - Implication of honeydew microflora. Presented at the Annual Symposium of the International Society of Chemical Ecology, Vilnius, Lithuania.

- Detrain, C., **Fischer, C.**, Lognay, G., Diez, L., Haubruge, E., Prieur, J., & Verheggen, F. (2012, August 27). Choosing an aphid partner: a matter of taste and smell. Presented at EUROIUSSI2012 - Combined European IUSSI Sections Meeting, Tuscany, Italy.

- Verheggen, F., Leroy, P., **Fischer, C.**, Sabri, A., Heuskin, S., Thonart, P., Fleton, G., Detrain, C., Lognay, G., & Haubruge, E. (2012, February). Microorganisms from aphid honeydew attract natural enemies and tending ants. Presented at the Gordon Research Conference on Plant Volatiles, Ventura, United-States.
- **Fischer, C**, Lognay, G, Haubruge, E, Detrain, C, & Verheggen, F. (2012, February 10). Implication of honeydew microflora in ant-aphid mutualism. Poster presented at the 17th PhD Symposium on Applied and Biological Sciences, Leuven, Belgium.
- **Fischer, C**, Patris, G, Duriaux, A, Sablon, L, Sautot, L, Haubruge, E, Detrain, C, Lognay, G, & Verheggen, F. (2011, September). Ant-aphid mutualism - A question of microorganisms? Poster presented at "Ento11 - Reception, detection, deception – Meeting of the Royal Entomological Society", Chatham, United Kingdom.
- Verheggen, F, Leroy, P, **Fischer, C**, Sabri, A, Francis, F, Heuskin, S, Thonart, P, Felton, G, Detrain, C, Lognay, G, & Haubruge, E. (2011, August). Microorganisms from aphid honeydew attract natural enemies and tending ants. Presented at the 27th Annual meeting of the International Society of Chemical Ecology, Vancouver (Burnaby), Canada.
- **Fischer, C**, Sablon, L, Duriaux, A, Patris, G, Sautot, L, Haubruge, E, Detrain, C, Lognay, G, & Verheggen, F. (2011, June). Ant-aphid mutualism - How do ants locate aphid colonies? Poster presented at "Trends in Natural Products Research: A PSE Young Scientists Meeting", Kolymvari, Crete.
- **Fischer, C**, Sablon, L, Duriaux, A, Patris, G, Sautot, L, Haubruge, E, Detrain, C, Lognay, G, & Verheggen, F. (2011, May 11-12). Le mutualisme fourmis-pucerons – Comment les fourmis trouvent-elles les pucerons? Poster presented during the Essenscia week-end, Gembloux, Belgium. (Poster)
- Sablon, L, **Fischer, C**, Leroy, P, Detrain, C, Haubruge, E, Lognay, G, & Verheggen, F. (2011, March). Ant-aphid mutualism: impact of aphid semiochemicals on the foraging behaviour of ants. Poster presented at the Global Conference on Entomology (GCE 2011), Chiang Mai, Thailand.

Thématiques annexes

Publications

Journaux à comité de lecture

- Daems, F., Béra, F., Lorge, S., **Fischer, C.**, Brostaux, Y., Francis, F., Lognay, G., & Heuskin, S. (2016). Impact of climatic factors on the release of E-β-caryophyllene from alginate beads. *BASE,* 20(2). http://hdl.handle.net/2268/195175
- Hurdebise, Q.*, Tarayre, C.*, **Fischer, C.***, Colinet, G., Hiligsmann, S., & Delvigne, F. (2015). Determination of Zinc, Cadmium and Lead Bioavailability in Contaminated Soils at the Single-Cell Level by a Combination of Whole-Cell Biosensors and Flow Cytometry. *Sensors*. http://hdl.handle.net/2268/181705

  (**these authors have contributed equally to this manuscript*)
- Censier, F.*****, **Fischer, C.***, Chavalle, S., Heuskin, S., Fauconnier, M.-L., Bodson, B., De Proft, M., Lognay, G. & Laurent, P. (2014). Identification of 1-methyloctyl butanoate as the major sex pheromone component from females of the saddle gall midge, *Haplodiplosis marginata* (Diptera: Cecidomyiidae). *Chemoecology,* 24(6), 243-251

  *(*both authors have contributed equally to this manuscript)*
- Fassotte, B., **Fischer, C.**, Durieux, D., Lognay, G., Haubruge, E., Francis, F., & Verheggen, F. (2014). First evidence of a volatile sex pheromone in ladybeetles. *PLoS ONE*, 9(12).
- Fassotte, B., **Fischer, C.**, Durieux, D., Lognay, G., Haubruge, E., Francis, F., & Verheggen, F. (2014). A volatile sex pheromone in the invasive ladybeetle Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae). *Communications in Agricultural and Applied Biological Sciences*, 79(1), 79-81.
- Durieux, D., Fassotte, B., Vanderplanck, M., Brostaux, Y., **Fischer, C.**, Lognay, G., Haubruge, E., & Verheggen, F. (2014). Is conspecific substrate marking a long-term external memory of previously colonized overwintering sites in Harmonia axyridis? *Journal of Applied Entomology*, 138(5), 338-345.
- Durieux, D., Fassotte, B., Fischer, C., Lognay, G., Haubruge, E., & Verheggen, F. (2014). Is Contact Between Conspecifics Involved in the Cohesion of Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) Aggregations? *Journal of Insect Behavior*, 1-13.

- Censier, F., **Fischer, C.**, Chavalle, S., Heuskin, S., Fauconnier, M.-L., De Proft, M., Lognay, G., Laurent, P., & Bodson, B. (2015). 7. Lutte intégrée contre les ravageurs - 2.1 Suivi des populations de cécidomyie équestre, Haplodiplosis marginata, grâce au développement d'un piège à phéromone. In B., Watillon & B., Bodson (Eds.), *Livre Blanc Céréales* (Février 2015, pp. 7/4-7/5). Gembloux, Belgium: Gembloux Agro-Bio Tech.
- Durieux, D., Fassotte, B., Vanderplanck, Deneubourg, J.-L., **Fischer, C.**, Lognay, G., Haubruge, E., & Verheggen, F. (2013). Substrate Marking by an Invasive Ladybeetle: Seasonal Changes in Hydrocarbon Composition and Behavioral Responses. *PLoS ONE*, 8(4).
- Gueye, M. T., Seck, D., Diallo, A., Trisman, D., **Fischer, C.**, Barthelemy, J.-P., Wathelet, J.-P., & Lognay, G. (2013). Development of a performant method for glucocapparin determination in Boscia senegalensis Lam ex. Poir. : A study of the variability. *American Journal of Analytical Chemistry*, 4, 104-110.
- Durieux, D., **Fischer, C.**, Fassotte, B., Sloggett, J., Deneubourg, J.-L., Lognay, G., Haubruge, E., & Verheggen, F. (2013). Aggregation behaviour of Harmonia axyridis. *IOBC/WPRS Bulletin*, 94, 93-94.
- Cawoy, H., Mariutto, M., Henry, G., **Fischer, C.**, Vasilyeva, N., Thonart, P., Ongena, M. (2013). Plant Defense Stimulation by Natural Isolates of Bacillus Depends on Efficient Surfactin Production. *Molecular Plant-Microbe Interactions*, 27(2), 87–100.
- Durieux, D, **Fischer, C**, Deneubourg, J.-L, Brostaux, Y, Lognay, G, Vandereycken, A, Joie, E, Haubruge, E, & Verheggen, F. (2012). Study of the factors involved in the aggregation of Harmonia axyridis Pallas (Coleoptera : Coccinellidae). *Communications in Agricultural and Applied Biological Sciences, 77*(1), 101-104.
- Durieux, D., **Fischer, C.**, Brostaux, Y., Sloggett, J., Deneubourg, J.-L., Vandereycken, A., Joie, E., Wathelet, J.-P., Lognay, G., Haubruge, E., & Verheggen, F. (2012). Role of long-chain hydrocarbons in the aggregation behaviour of Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae). *Journal of Insect Physiology,* 58:801-807.
- **Fischer, C**, Laurent, P, Heuskin, S, Durieux, D, Haubruge, E, Verheggen, F & Lognay, G. (2012). Fast-GC quantification of harmonine, the major defense alkaloid of the multicolored Asian ladybird Harmonia axyridis (Pallas). *American Journal of Analytical Chemistry, 3*, 67-75.

Journaux sans comité de lecture

- Alabi, T., Brostaux, Y., Grigorescu, A., **Fischer, C.**, Thonart, P., Lognay, G., Haubruge, E., Francis, F., & Verheggen, F. (2014). Microorganism-associated semiochemicals reduce the size of aphid populations in potato fields. *Revue des Régions Arides*, 35. http://hdl.handle.net/2268/178029

Communications

- Fassotte, B., **Fischer, C.**, Durieux, D., Lognay, G., Haubruge, E., Francis, F., & Verheggen, F. (2015, May 13). First evidence of a volatile sex pheromone in Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae). Paper presented at Benefits and Risks of Exotic Biological Control Agents, Bornholm, Danemark.

- **Fischer, C.**, Tarayre, C., Zune, Q., De Weever, H., Van Hecke, W., & Delvigne, F. (2014, September 09). Succinic acid production in a biofilm bioreactor. Poster presented at the 10th European Symposium on Biochemical Engineering Sciences and 6th International Forum on Industrial Bioprocesses, Lille, France.

- Tarayre, C., Hurdebise, Q., **Fischer, C.**, Colinet, G., Buysse, J., Michels, E., Meers, E., & Delvigne, F. (2014, September 09). BioRefine Project: Detection of bioavailability of Metallic Trace Elements in soils by the use of microbial biosensors. Poster presented at the 10th European Symposium on Biochemical Engineering Sciences and 6th International Forum on Industrial Bioprocesses, Lille, France.

- Tarayre, C., **Fischer, C.**, De Clercq, L., Michels, E., Meers, E., Buysse, J., Delvigne, F., & Thonart, P. (2014, June 05). Biorefine: Recovery of nutrients and metallic trace elements from different wastes by chemical and biochemical processes. Presented at 10th International Conference on Renewable Resources and Biorefineries.

- Fassotte, B., **Fischer, C.**, Durieux, D., Lognay, G., Haubruge, E., Francis, F., & Verheggen, F. (2014, October). Une phéromone sexuelle chez la coccinelle asiatique, Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae). Presented at Premières rencontres nationales des Coccinellistes, Angers, France

- Fassotte, B., **Fischer, C.**, Durieux, D., Lognay, G., Haubruge, E., Francis, F., & Verheggen, F. (2014, April 02). First evidence of a volatile sex pheromone in the invasive Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae). Presented at *Entomophagistes* 2014, Louvain-la-Neuve, Belgique.

- Fassotte, B., **Fischer, C.**, Durieux, D., Vandereycken, A., Lognay, G., Haubruge, E., Francis, F., & Verheggen, F. (2013, October). First Evidence of a Volatile Sex Pheromone in Harmonia axyridis (Pallas). Poster presented at the 2nd International Congress on Biological Invasions, Qingdao, China.
- Fassotte, B., **Fischer, C.**, Durieux, D., Vandereycken, A., Lognay, G., Haubruge, E., Francis, F., & Verheggen, F. (2013, October 19). Une phéromone sexuelle chez la coccinelle asiatique, Harmonia axyridis (Pallas). Poster presented at the 11ème journée entomologique de Gembloux, Gembloux, Belgique.
- Fassotte, B., **Fischer, C.**, Durieux, D., Lognay, G., & Verheggen, F. (2013, September 10). First evidence of a volatile sex pheromone in the invasive Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae). Presented at Ecology of Aphidophaga 12, Belgrade, Serbia.
- Heuskin, S., **Fischer, C**. *et al.*, (2012). Development of semiochemical slow-release formulations as biological control devices. Poster presented at the 28th International Society of Chemical Ecology Annual Meeting, Vilnius, Lithuania
- Durieux, D., **Fischer, C.**, Fassotte, B., Brostaux, Y., Lognay, G., Haubruge, E., & Verheggen, F. (2012, July). Flexible aggregative behavior of Harmonia axyridis according to the freshness of area marking in overwintering sites. Poster presented at the 28th International Society of Chemical Ecology Annual Meeting, Vilnius, Lithuania.
- Durieux, D., Fassotte, B., Vanderplanck, M., **Fischer, C.**, Lognay, G., Francis, F., Haubruge, E., & Verheggen, F. (2012, August). Behavioral response of Harmonia axyridis towards their footprints according to their physiological state. Poster presented at 24th International Congress of Entomology, Daegu, Korea.
- Durieux, D., **Fischer, C.**, Deneubourg, J.-L., Brostaux, Y., Lognay, G., Vandereycken, A., Joie, E., Haubruge, E., & Verheggen, F. (2012, May 22). Factors involved in the aggregation of Harmonia axyridis Pallas. Presented at 64th International Symposium on Crop Protection, Ghent, Belgium.
- Durieux, D, **Fischer, C**, Deneubourg, J.-L, Brostaux, Y, Lognay, G, Vandereycken, A, Joie, E, Haubruge, E, & Verheggen, F. (2012). Study of the factors involved in the aggregation of Harmonia axyridis Pallas (Coleoptera : Coccinellidae). Presented at the 17th PhD Symposium on Applied and Biological Sciences, Leuven, Belgium.

- Durieux, D, **Fischer, C**, Deneubourg, J.-L, Brostaux, Y, Lognay, G, Vandereycken, A, Joie, E, Haubruge, E, & Verheggen, F. (2011, July 27). Chemical and physical factors involved in the aggregation behaviour of Harmonia axyridis Pallas. Poster presented at the 27th meeting of the International Society of Chemical Ecology, Burnaby, Canada.
- Durieux, D, **Fischer, C**, Lognay, G, Deneubourg, J.-L, Vandereycken, A, Joie, E, Haubruge, E, & Verheggen, F. (2011, June 12). Implication of hydrocarbons in the aggregation behaviour of Harmonia axyridis Pallas (Coleoptera : Coccinellidae). Presented at" Trends in Natural Products Research: A PSE Young Scientists Meeting", Kolymvari, Crète. (Poster)
- **Fischer, C**, Sibret, V, Laurent, P, Verheggen, F, & Lognay, G. (2010, June 02). Evolution of Harmonia axyridis (Pallas) alkaloid protection during the year in relation with insect color. Poster presented at the "Insect Chemical Ecology 2010" meeting, State College, PA.
- **Fischer, C**, Sibret, V, Laurent, P, Haubruge, E, Verheggen, F, & Lognay, G. (2010, May 05). Evolution of Harmonia axyridis (Pallas) alkaloid protection during the year. Poster presented at the One-Day Symposium on Chemical Entomology, Gembloux, Belgium.
- **Fischer, C**, Heuskin, S, Laurent, P, Marlet, C, Durieux, D, Verheggen, F, & Lognay, G. (2010, January). Harmonia axyridis (Pallas) secondary metabolites quantification in relation with aposematism : Part I : Harmonine quantification. Poster presented at the Eleventh International Symposium on Hyphenated Techniques in Chromatography (HTC-11), Bruges, Belgium.
- **Fischer, C**, Heuskin, S, Marlet, C, Durieux, D, Laurent, P, Verheggen, F, & Lognay, G. (2010, January). Harmonia axyridis (Pallas) secondary metabolites quantification in relation with aposematism : Part II : Pyrazine quantification. Poster presented at the Eleventh International Symposium on Hyphenated Techniques in Chromatography (HTC-11), Bruges, Belgium.

DISTINCTIONS ET FINANCEMENTS

Novembre 2015	Colauréat du prix scientifique de l'Institut Phytofar (Belgique)
2013 - 2017	Membre du Collège des Alumni de l'Académie royale de Belgique
Juillet 2012	*Best Student Presentation Award*, Symposium de l' *International Society of Chemical Ecology (ISCE)*, Vilnius (Lituanie)
Mars 2012	Bourse de voyage du Fonds Agathon de Potter (Académie Royale des sciences, des lettres et des beaux-arts de Belgique)
Décembre 2011	Bourse de doctorat du Fonds pour la formation à la Recherche dans l'Industrie et dans l'Agriculture (FRIA)(renouvellement)
Septembre 2011	Prix du meilleur poster (seconde place), *Royal Entomological Society's meeting*, Chatham (UK)
Décembre 2009	Bourse de doctorat du Fonds pour la formation à la Recherche dans l'Industrie et dans l'Agriculture (FRIA)