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A multitrophic approach to the dynamics of insect herbivores

Experiments with plants, predators and insect symbionts

Enric Frago - CIRAD CBGP
Centre de Coopération Internationale en Recherche Agronomique pour le Développement
Centre de Biologie pour la Gestion des Populations

Defended the 08/12/2023 before the jury:
Alison Duncan - CNRS, ISEM, Montpellier (Examinatrice)
Elisa Thebault - CNRS, iEES, Paris (Rapportrice)
Fabrice Vavre - CNRS, IBBE, Lyon (Rapporteur)
Elodie Vercken - INRAE, ISA, Sophia-Antipolis (Rapportrice)
Christoph Vorburger - ETH, Zurich (Examinateur)
Christine Meynard - INRAE, CBGP, Montpellier
(Examinatrice & Présidente)

" Je déclare avoir respecté, dans la conception et la rédaction de ce mémoire d'HDR, les valeurs et principes d'intégrité scientifique destinés à garantir le caractère honnête et scientifiquement rigoureux de tout travail de recherche, visés à l'article L.211-2 du Code de la recherche et énoncés par la Charte nationale de déontologie des métiers de la recherche et la Charte d'intégrité scientifique de l'Université de Montpellier. Je m'engage à les promouvoir dans le cadre de mes activités futures d'encadrement de recherche."

On vit un moment de bouleversement en France. J'aimerais dédier ce travail aux personnes qui se battent contre la réforme globale des retraites.

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PROLOGUE

My scientific path and a few definitions

Insect herbivores and their host plants form the base of some of the most complex communities of terrestrial ecosystems. Understanding such **complexity** has fuelled research by ecologists and has been driven by both curiosity and need. Curiosity to understand and the need to manage pests. Insect herbivores feed on **plants** but they are also fed by their **natural enemies**. These three level networks are far from simple and are dominated by intricate interactions. If **microbial associates** of these three trophic levels are considered, the system becomes even more complex. As I will explain in this document, most of my research has been devoted to understanding this complexity, and to place it in an applied framework to control pest species. I haven't done this journey alone. Since the beginning of my PhD I have collaborated with many scientists including BSc, PhD, and MSc students, permanent researchers, and postdocs.

During my PhD I studied the population dynamics of the moth *Euproctis chrysorrhoea* in the Iberian Peninsula. A highly polyphagous pests that mostly feeds on forest trees. This species was little known in Europe. We had some references mostly from Russia, France and Italy, with little knowledge in Spain and Portugal. I was obliged to read my first papers in French and Italian, and to find a translator for the papers in Russian. We wanted to understand how the dynamics of this pest was determined by natural enemies. We particularly focused on **parasitoids**, a group of insects (mostly wasps and flies) that has fascinated many generations of entomologists (me included) due to their spectacular diversity of species, behaviours and life histories. Parasitoids deposit an egg in, on, or in the vicinity of their hosts, which develops into a larva that eventually kills the host. Our study was mostly observational, and based on sampling and rearing the different stages of the moth. We obtained a total of 26 parasitoids, many of them new for the parasitoid complex of the moth *E. chrysorrhoea.* We mostly found primary parasitoids that thrived attacking the moth, but many were secondary parasitoids or **hyperparasitoids** that attacked the primary ones. We even found a tertiary parasitoid that lived upon secondary ones, a complex set of Matryoshka dolls that made me realise the complexity of interactions that can arise from a single basal resource. It was clear that species dynamics could not be understood in isolation, and was the beginning of my interest for **community ecology** approaches to understand animal ecology. Since the core of my thesis was observational, we were forced to speculate about the mechanisms behind the dynamics of this moth. Moving from speculation to actual proof requires **experiments** and *E. chrysorrhoea* was not a good model for this. This species has a long development time, one year to complete a cycle, and is the third most toxic insect species in Europe. Its hairs contain a toxic protein that can trigger strong urticating reactions, all stages from egg to adults containing such hairs. Performing experiments with this species was challenging, and I therefore decided to change to aphids as my experimental model by doing a couple of postdocs mostly working with the aphid *Acyrthosiphon pisum.* This aphid is a model species for the study of insect interactions with plants, insect natural enemies and insect symbionts. In my first postdoc in England, I performed experiments manipulating the presence and absence of different natural enemies. Getting inspiration from previous work done in the hosting laboratory I started my work on the role of **indirect interactions** in driving the dynamics of insect communities. Indirect interactions are interactions between species that occur through at least a third one. In this first postdoc I worked on how natural enemies can mediate interactions between different species of herbivorous insects, an **enemy-mediated indirect effect** known as **apparent competition**. If my PhD model needed seven months to undergo a generation, aphids did so in one week thus allowing me to study **long-term community dynamics** that occur over several insect generations. In my second postdoc in the Netherlands, I kept working with aphids, but I included in my research two new approaches. First I started my work on how changes in plant physiology brought by herbivory modulates interactions between herbivores, a **plant-mediated indirect effect**. I also started my experimental work on the impact that aphid symbionts had on the complex interactions I mentioned above. I was particularly interested in **defensive symbionts**, symbionts that protect their hosts against natural enemies and parasitoids in particular. It was during this second postdoc that I started collaborating with many early career scientists including BSc, PhD and MSc students. I did this postdoc in a large laboratory, where postdocs were usually supervised at least one MSc and several BSc students per year. PhD students were also eager for collaborations with more experienced postdocs, and even if I was never a formal part of the supervising team, I was involved in the making of many PhD chapters. During that time I still kept my links with the laboratory where I did my PhD, and I was involved in the co-supervision of the PhD of Laia Fontan-Bria.

I 2016 I got a permanent job as researcher at Cirad. My first job at Cirad was at Reunion island, a French overseas territory. I started a program on **experimental community ecology** to understand the dynamics of pest species and their natural enemies in commercial greenhouses. At that time a small company was starting to **mass produce natural enemies** to release them in greenhouses and there was a need to better understand the ecology of these species. By selecting pest species found naturally in greenhouses, we set-up communities in the laboratory and we exposed them to different enemies produced by this company. We performed experiments that included behavioural tests, field observations, and tests in **population cages** to follow the long-term dynamics of these complex communities. We were particularly interested in assessing whether enemies engaged in **intraguild predation**, which occurs when two natural enemies that feed on a common prey also predate on each other. Our work focussed on testing fundamental community ecology questions, while at the same time **providing advice** on the use and efficiency of the different natural enemies used. This work was done in collaboration with laboratory assistants, permanent scientists, but also with several MSc students, and two PhD students that I co-supervised, Niry Dianzinga and Karim Tighiouart. The data and experience that we accumulated during that time was key to starting my research line when I moved from Reunion to Montpellier. These experiences were also important as they set the ground to secure funding for the research project that I will coordinate from 2023 to 2026. In this project the same keywords mentioned above reappear: complexity, indirect-effects, natural enemies, long-term community dynamics, intraguild predation, greenhouses and mass production of natural enemies. A new PhD is also expected and I hope this time I will be the main supervisor.

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(in chronological and/or alphabetical order)

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RÉSUMÉ EN FRANÇAIS

PROLOGUE : MON PARCOURS SCIENTIFIQUE ET QUELQUES DÉFINITIONS.

Les insectes herbivores et leurs plantes hôtes forment la base des communautés les plus complexes des écosystèmes terrestres. La compréhension de cette complexité a alimenté les recherches des écologistes, motivées à la fois par la curiosité et le besoin. La curiosité de comprendre et la nécessité de lutter contre les ravageurs. Les insectes herbivores se nourrissent de plantes, mais ils sont également attaqués par leurs ennemis naturels. Ces réseaux à trois niveaux sont loin d'être simples, et ils sont dominés par des interactions complexes. Si l'on ajoute les microbes associés à ces trois niveaux trophiques, le système devient encore plus complexe. Comme je l'expliquerai dans ce manuscrit, la plupart de mes recherches ont été consacrées à la compréhension de cette complexité et à son intégration dans un cadre appliqué à la lutte biologique contre les espèces nuisibles. Je n'ai pas fait ce voyage seul. Depuis le début de mon doctorat, j'ai collaboré avec de nombreux scientifiques, notamment des étudiants en licence et en maîtrise, des doctorants et post-doctorants ainsi que des chercheurs permanents.

Au cours de mon doctorat, j'ai étudié la dynamique des populations du papillon de nuit *Euproctis chrysorrhoea* dans la péninsule ibérique. Il s'agit d'un ravageur très polyphage qui se nourrit principalement d'arbres forestiers, assez peu connu en Europe. Mes travaux se sont basés sur quelques références provenant principalement de Russie, de France et d'Italie, avec peu de connaissances en Espagne et au Portugal. J'ai dû lire mes premiers articles en français et en italien, et trouver un traducteur pour les articles en russe. Nous voulions comprendre comment la dynamique de ce ravageur était déterminée par les ennemis naturels. Nous nous sommes particulièrement intéressés aux parasitoïdes, un groupe d'insectes (principalement des guêpes et des mouches) qui a fasciné de nombreuses générations d'entomologistes (moi y compris) en raison de leur spectaculaire diversité spécifique, de comportements et d'histoires de vie. Les parasitoïdes déposent un œuf dans, sur ou à proximité de leur hôte, qui se développe en larve qui finit par tuer l'hôte. Notre étude a principalement reposé sur l'observation, l'échantillonnage et l'élevage des différents stades de ce papillon de nuit. Nous avons ainsi récolté un total de 26 espèces différentes de parasitoïdes. Nous avons trouvé à la fois des parasitoïdes primaires, attaquant directement le papillon, mais également des parasitoïdes secondaires, ou hyperparasitoïdes, attaquant les parasitoïdes primaires. Nous avons même trouvé un parasitoïde tertiaire qui exploitait des parasitoïdes secondaires. Cet ensemble complexe de poupées Matryoshka m'a fait prendre conscience de la complexité des interactions qui peuvent naître d'une seule ressource basale. Il était clair que la dynamique des espèces ne pouvait pas être comprise de manière isolée, et c'est ainsi que j'ai commencé à m'intéresser aux approches d'écologie des communautés afin de mieux comprendre les dynamiques des populations animales. Le cœur de ma thèse étant basé sur l'observation, nous avons été contraints de spéculer sur les mécanismes à l'origine de la dynamique de ce papillon de nuit. Passer de la spéculation à la preuve concrète nécessite des expériences, et faire cela n'était pas possible avec *E. chrysorrhoea.* Cette espèce a un long temps de développement, un an pour compléter son cycle, et est la troisième espèce d'insectes la plus toxique en Europe. Ses poils contiennent une protéine toxique qui peut déclencher de fortes réactions urticantes, tous les stades, de l'œuf à l'adulte, contenant de tels poils. Il était difficile de réaliser des expériences avec cette espèce et je me suis donc tourné vers les pucerons en effectuant deux stages post-doctoraux, principalement sur le puceron du pois *Acyrthosiphon pisum.* Ce puceron est une espèce modèle pour l'étude des réseaux d'interactions entre les insectes phytophages, leurs plantes-hôtes, leurs ennemis naturels et leurs symbiotes. Lors de mon premier postdoc en Angleterre, j'ai réalisé des expériences manipulant la présence/absence de différents ennemis naturels. En m'inspirant de travaux antérieurs réalisés dans le laboratoire d'accueil, j'ai commencé à travailler sur le rôle des interactions indirectes dans la dynamique des communautés d'insectes. Les interactions indirectes sont des interactions entre espèces qui se produisent par l'intermédiaire d'au moins une troisième espèce. Dans ce premier postdoc, j'ai travaillé sur la façon dont les ennemis naturels peuvent servir de médiateurs aux interactions entre différentes espèces d'insectes herbivores, un effet indirect connu sous le nom de compétition apparente. Lors de mon second postdoc aux Pays-Bas, j'ai continué de travailler sur les pucerons, mais j'ai intégré deux nouvelles approches à mes recherches. Tout d'abord, j'ai commencé à travailler sur la façon dont les changements dans la physiologie des plantes provoqués par les insectes phytophages modulent les interactions entre les herbivores, un effet indirect médié par les plantes. J'ai également commencé mon travail expérimental sur l'impact des symbiotes des pucerons sur les interactions complexes mentionnées ci-dessus. Je me suis particulièrement intéressé aux symbiotes défensifs, c'est-à-dire aux symbiotes qui protègent leurs hôtes contre les ennemis naturels, et en particulier les parasitoïdes. C'est au cours de ce deuxième postdoc que j'ai commencé à collaborer avec de nombreux scientifiques en début de carrière, y compris des étudiants en licence, en maîtrise et en doctorat. J'ai effectué ce postdoc dans un laboratoire où les postdocs étaient souvent impliqués dans la supervision d'au moins un étudiant en maîtrise et plusieurs étudiants en licence par an. Les doctorants étaient également désireux de collaborer avec des postdocs plus expérimentés, et même si je n'ai jamais fait officiellement partie de l'équipe de supervision, j'ai participé à l'élaboration de nombreux chapitres de thèse. Pendant cette période, j'ai gardé des liens avec le laboratoire où j'ai effectué mon doctorat, et j'ai participé à la co-supervision du doctorat de Laia Fontan-Bria.

Après ces deux postdocs, j'ai obtenu un emploi permanent de chercheur au Cirad. Mon premier poste au Cirad était à l'île de la Réunion, un territoire français d'outre-mer. J'ai démarré un programme en écologie expérimentale des communautés pour comprendre la dynamique des espèces de ravageurs et de leurs ennemis naturels dans les serres commerciales. À l'époque, une petite entreprise commençait à produire en masse des ennemis naturels pour les lâcher dans les serres, et il était nécessaire de mieux comprendre l'écologie de ces espèces. A partir d’espèces de ravageurs présentes naturellement dans les serres, j’ai construit des communautés au laboratoire et nous les avons exposées à différents ennemis produits par cette entreprise. Nous avons réalisé des expériences comprenant des observations sur le terrain ainsi que des tests comportementaux et des suivis de dynamique des populations en conditions contrôlées au laboratoire afin de mieux comprendre la dynamique à long terme (i.e. sur plusieurs générations) de ces communautés complexes. Je me suis particulièrement intéressé à la prédation intraguilde, qui se produit lorsque deux ennemis naturels qui se nourrissent d'une proie commune se nourrissent également l'un de l'autre. Ces expériences ont permis d'étudier des questions fondamentales en écologie des communautés, tout en fournissant des conseils sur l'utilisation et l'efficacité des différents ennemis naturels fournis par l’entreprise partenaire. Ce travail a été réalisé en collaboration avec des assistants de laboratoire, des scientifiques permanents, mais aussi avec plusieurs étudiants en maîtrise et deux étudiants en doctorat que j'ai co-encadrés, Niry Dianzinga et Karim Tighiouart. Les données et l'expérience que nous avons accumulées pendant cette période ont été essentielles pour définir ma thématique de recherche lorsque j'ai quitté la Réunion pour Montpellier. Ces expérimentations ont également été importantes car elles m’ont permis d'obtenir un financement ANR pour un projet de recherche que je coordonnerai de 2023 à 2026. Dans ce projet, les mêmes thématiques mentionnées ci-dessus apparaissent : complexité, effets indirects, ennemis naturels, dynamique des communautés à long terme, prédation intraguilde, serres et production de masse d'ennemis naturels. Un doctorat est également prévu, et j'espère que cette fois-ci je serai le directeur.

CONTEXTE GÉNÉRAL

Les insectes phytophages sont à la base de certaines des communautés les plus complexes de la planète, et la compréhension de leur dynamique intéresse les écologistes depuis longtemps. Cet intérêt repose à la fois sur une curiosité de comprendre la nature, mais aussi sur la nécessité de gérer les ravageurs agricoles et forestiers. Les insectes herbivores se trouvent "in between the devil and the deep blue sea”, car ils doivent faire face aux défenses anti-herbivores des plantes, tout en évitant ou en résistant à leurs ennemis naturels. Ces deux forces, également appelées "top-down" (effets de l'ennemi sur les herbivores) et "bottom-up" (effets de la plante sur les herbivores), agissent simultanément, et le débat sur leur importance relative dans la détermination de la dynamique des herbivores est aussi ancien que irrésolu. La dynamique des populations d'herbivores peut être déterminée par les défenses des plantes, les ennemis naturels ou les deux. Le facteur dominant est toutefois spécifique à chaque espèce et à chaque système, et même au sein d'un même système, l'équilibre entre ces forces est extrêmement variable dans le temps et dans l'espace. Si ces interactions complexes rendent difficile la compréhension de la dynamique des herbivores, la question est devenue encore plus compliquée avec la récente prise de conscience de l'importance des symbiotes dans la biologie des insectes. Cette prise de conscience a été possible grâce aux progrès technologiques, et à la biologie moléculaire en particulier, qui ont rendu l'étude des microorganismes plus ouverte et plus accessible aux organismes non modèles. Les symbiotes d'insectes sont capables de faciliter l'alimentation des herbivores sur les plantes, mais aussi de protéger leurs hôtes contre les ennemis naturels, de sorte qu'ils sont désormais considérés comme faisant partie intégrante des réseaux trophiques des herbivores.

INTERACTIONS ENTRE LES INSECTES HERBIVORES ET LES PLANTES

Les premières études sur les effets “bottom-up” des plantes sur les herbivores ont déjà identifié qu'en plus de la cellulose et de la lignine, les plantes mélangent ces deux éléments de base avec un cocktail complexe de métabolites, connus sous le nom de métabolites secondaires. Il est désormais reconnu que les composés secondaires des plantes modulent les interactions, non seulement avec les herbivores, mais aussi avec les mutualistes tels que les pollinisateurs, les plantes compétitrices et les facteurs de stress abiotiques. La production de ces composés est toutefois coûteuse sur le plan métabolique, ainsi en l'absence d'herbivores, les plantes ont développé des défenses induites. À l'instar du système immunitaire des animaux, les défenses induites ne sont déclenchées qu'en cas d'attaque d'un herbivore ou d'un agent pathogène, et elles sont hautement spécifiques afin d'adapter la résistance à l'agresseur particulier qui déclenche la réponse. Les défenses agissent en fonction de la détection de types spécifiques de dommages causés par l'alimentation et/ou de composés présents dans les sécrétions orales des herbivores. Les phytohormones, qui sont à la base des voies de transduction des signaux conduisant à l'expression des gènes de défense, sont l'un des moyens utilisés par les plantes pour affiner ces défenses. Plusieurs phytohormones sont impliquées dans les défenses induites des plantes, mais les plus étudiées sont l'acide jasmonique, l'acide salicylique et l'éthylène. Lorsque les plantes sont attaquées par plusieurs phytophages, les réponses spécifiques pour contrer une espèce donnée peuvent moduler la résistance de la plante à une autre espèce. Les herbivores qui partagent des plantes-hôtes interagissent donc indirectement par le biais de changements dans la physiologie des plantes.

Les défenses des plantes impliquent également l'émission de substances volatiles végétales qui attirent les ennemis des ravageurs, connues sous le nom de *Herbivore Induced Plant Volatil*es. Lors d'une attaque d'herbivore, ces composés organiques volatils sont créés ou, s'ils sont présents de manière constitutive, leur concentration augmente. Ces composés volatils ont un effet positif sur les plantes en réduisant leur consommation par les herbivores. Ce système de défense des plantes a suscité beaucoup d'intérêt au cours des 30 dernières années, notamment de la part des écologistes, mais aussi des entomologistes spécialistes du biocontrôle, car les plantes peuvent être sélectionnées ou modifiées pour mieux attirer les ennemis des ravageurs. Mon principal attrait pour les défenses végétales porte sur leur capacité à moduler les interactions entre les espèces, et donc à structurer les communautés d'herbivores. Plus précisément, les changements dans la physiologie des plantes provoqués par l'action des pohytophages auront des conséquences pour les autres espèces qui prospèrent sur cette même plante. Si des substances volatiles sont impliquées, des interactions peuvent même se produire entre des herbivores se nourrissant de plantes différentes.

INTERACTIONS COMPLEXES ENTRE LES ENNEMIS NATURELS DES INSECTES

Le fait que les plantes aient évolué vers un processus aussi complexe que l'émission de substances volatiles pour renforcer le contrôle top-down des herbivores, montre toute l’importance que revêtent, pour les végétaux, les ennemis naturels des espèces phytophages. Les introductions involontaires d'insectes dans des habitats exotiques constituent des expériences naturelles qui ont révélé l'importance de ces alliés. Lorsque des espèces envahissantes colonisent des nouvelles zones géographiques, elles se développent souvent dans des écosystèmes dépourvus de leurs ennemis naturels, ce qui leur permet de proliférer, avec des conséquences parfois dramatiques pour les espèces autochtones ou pour les cultures agricoles. Les exemples de programmes classiques de lutte biologique dans lesquels les espèces envahissantes ont été contrôlées en transférant leurs ennemis naturels de la zone d'origine vers la zone exotique, fournissent également de bonnes preuves du potentiel des ennemis naturels pour la suppression des espèces invasives. Les interactions entre les phytophages et leurs ennemis naturels sont toutefois rarement simples et les solutions miracles pour lutter contre les ravageurs sont rares. De nombreux programmes de biocontrôle échouent parce que les réseaux trophiques sont fortement imbriqués les uns dans les autres. Plusieurs ennemis naturels peuvent être nécessaires pour contrôler un ravageur, les ennemis naturels peuvent s’éliminer mutuellement au lieu de s’attaquer aux ravageurs par prédation intraguilde, ou ils peuvent se déplacer vers d'autres hôtes qui ne sont pas des ravageurs. En écologie des communautés, il est donc important de bien choisir l’approche que l’on souhaite utiliser pour mettre en place une stratégie de biocontrôle efficace. Une partie importante de mes recherches a été consacrée à l'apport de connaissances écologiques fondamentales, utiles pour comprendre la dynamique des communautés d'herbivores, mais aussi pour contrôler les ravageurs. Développer des principes généraux pour mettre en œuvre un biocontrôle réussi ne serait pas possible sans l’écologie théorique, mais cette théorie doit en retour être nourrie par la pratique du biocontrôle. Malgré de nombreuses avancées théoriques et une masse croissante d'informations disponibles, les praticiens du biocontrôle ne connaissent pas toujours l'importance des interactions indirectes complexes telles que la prédation intraguilde ou la compétition apparente. Cette appréciation est importante pour mieux comprendre la dynamique des ravageurs car, que les insectes vivent dans des forêts vierges ou sur des terres agricoles, l'impact des ennemis naturels peut rarement être prédit à partir des interactions entre les espèces prises deux par deux. Une part importante de mes recherches actuelles est consacrée à cette compréhension.

LES SYMBIOTES DES HERBIVORES DÉTERMINENT LES INTERACTIONS AVEC LES PLANTES ET LES ENNEMIS NATURELS

La plupart des réseaux trophiques terrestres peuvent être décrits comme tripartites, c'est-à-dire constitués par les plantes, les phytophages et les consommateurs de niveau supérieur. Cette représentation est désormais reconnue comme incomplète si les microorganismes associés à ces trois niveaux trophiques ne sont pas pris en compte. Cette prise de conscience n'aurait pas été possible sans les progrès technologiques, en particulier en biologie moléculaire, qui rendent l'étude des microbiotes de moins en moins onéreuse et applicable aux organismes qui ne sont pas, à priori, des espèces modèles. Je me suis particulièrement intéressé aux symbiotes des insectes phytophages, qui sont désormais reconnus comme des acteurs clés de la dynamique et de la structure des réseaux trophiques incluant des insectes. La plupart des insectes ont besoin d'un microbiote pour pouvoir se nourrir de plantes, mais il existe aussi de nombreuses autres stratégies qui permettent la phytophagie. Les insectes suceurs de sève comme les pucerons et les aleurodes, par exemple, s'appuient sur des associations obligatoires, c'est-à-dire des symbiotes nécessaires à la survie de l'hôte. La sève qui circule dans le phloème des plantes est de mauvaise qualité nutritionnelle et manque de nombreux acides aminés essentiels que ces groupes d'insectes se procurent auprès de bactéries. D'autres groupes, comme les phasmes, les thrips et les orthoptères établissent principalement des associations transitoires et/ou s'appuient sur des gènes microbiens qu'ils ont incorporés par transfert horizontal de gènes. Chez les insectes holométaboles, comme les lépidoptères, les coléoptères et les diptères, les symbiotes doivent persister pendant la métamorphose. Cette transition implique souvent des associations transitoires acquises dans l'environnement à chaque génération, même si une transmission verticale ou pseudo-verticale existe chez de nombreuses espèces. Les insectes utilisent également des services microbiens pour contourner les différentes modalités de défense que les plantes déploient pour contrer les attaques des herbivores. On sait depuis longtemps que les insectes symbiotes contribuent à la détoxification des métabolites secondaires des plantes. Comme l'ont montré certaines de mes recherches, les symbiotes peuvent aussi aider leurs hôtes en entravant l'induction des défenses des plantes.

Les dernières décennies ont également permis de faire des découvertes passionnantes sur la manière dont les symbiotes peuvent défendre leurs hôtes contre les ennemis naturels. L'utilisation généralisée de symbiotes protecteurs par les animaux se retrouve dans tous les grands taxons, des éponges aux vertébrés, en passant par les mollusques, les crustacés et, bien sûr, les insectes. Des symbiotes défensifs ont été découverts dans de nombreux groupes d'insectes. L'un des exemples les plus étudiés de symbiote défensif chez les insectes est probablement le symbiote de différents pucerons *Hamiltonella defensa*, sur lequel j'ai beaucoup travaillé. Cette bactérie protège ses hôtes des parasitoïdes par la production de toxines. *H. defensa* est l'un des nombreux symbiotes facultatifs des pucerons. Les symbiotes facultatifs ne sont pas nécessaires à la survie de l'hôte mais fournissent des avantages sous certaines conditions. *H. defensa*, par exemple, peut être bénéfique en présence d'ennemis naturels, mais devient coûteux en leur absence. Je me suis particulièrement intéressé à ces types de symbioses et j'ai étudié leurs effets sur la dynamique des communautés complexes d'insectes et sur la manipulation des défenses des plantes.

PROJET DE RECHERCHE

Mon principal projet actuel est le projet EnemyCocktail "Designing natural enemy cocktails for a better biocontrol" que j'ai commencé à coordonner en janvier. Ce projet est financé par l'Agence nationale de la recherche française et durera jusqu'en 2026. L'idée principale de ce projet est d'adopter une approche d'écologie des communautés pour améliorer le biocontrôle inondatif (c'est-à-dire basé sur l'élevage et le lâcher en masse d'ennemis naturels) en utilisant des combinaisons d'ennemis naturels, ou cocktails. Ce projet est un effort collectif de scientifiques et des acteurs du biocontrôle en France, en Angleterre et en Belgique. Nous collaborerons notamment avec la société Biobest qui produit en masse des ennemis pour des lâchers dans des serres commerciales. Une contrainte importante pour le succès du biocontrôle est qu'il repose sur une connaissance écologique approfondie des interactions entre les espèces et, comme je l'ai déjà montré dans les pages précédentes, son succès est souvent entravé par la nature complexe des interactions écologiques. Un défi majeur du biocontrôle est de trouver les meilleurs cocktails d'ennemis naturels pour contrôler les ravageurs en minimisant les interactions antagonistes entre eux. Ce défi est particulièrement important dans le cas de la lutte biologique par inondation, car les lâchers en masse concentrent les ennemis et favorisent les interactions entre les espèces, mais aussi parce que les lâchers d'un seul ennemi sont rarement suffisants pour lutter contre les ravageurs. Dans ce contexte, les connaissances en écologie des communautés sont essentielles à la réussite d'un programme de biocontrôle car, que ce soit en serre ou dans les écosystèmes naturels, l'impact des ennemis naturels sur les proies peut rarement être prédit à partir des interactions entre espèces deux à deux.

Le projet utilisera la théorie écologique sur la relation entre la diversité des ennemis naturels et le biocontrôle, c'est-à-dire la relation biodiversité-biocontrôle. Cette relation émerge à travers "l'effet de complémentarité", qui peut être atténué par des "effets antagonistes". Dans les assemblages d'ennemis complémentaires, différentes espèces remplissent différentes fonctions ou répartissent leurs ressources, par exemple en exploitant des proies à différents stades ou à différentes échelles spatio-temporelles. La complémentarité réduit la compétition interspécifique entre les ennemis et facilite ainsi la coexistence des ennemis naturels. L'effet positif de la diversité des ennemis naturels sur le biocontrôle peut toutefois être atténué par des "effets antagonistes" entre les ennemis. Dans les communautés d'arthropodes, ces effets sont souvent dus à la prédation intraguilde. Malgré des décennies de recherche sur la façon dont la biodiversité des ennemis et la prédation intraguilde influencent la dynamique des herbivores, les expériences où le rôle de ces deux forces opposées a été testé ensemble sont encore très limitées. Malgré des prédictions vérifiables sur le rôle de la complémentarité et de la prédation intraguilde dans la relation biodiversité-biocontrôle, les preuves concluantes qui testent le rôle relatif de ces deux mécanismes opposés font encore défaut. Nous manquons en particulier d'informations sur la manière dont ces deux mécanismes agissent en combinaison pour conduire la dynamique des communautés de multi-prédateurs, une connaissance nécessaire pour mettre en œuvre efficacement le biocontrôle. Il est important de séparer ces deux forces opposées car, dans le biocontrôle appliqué, de nombreux ennemis sont généralistes et agissent souvent comme des prédateurs intraguildes en se nourrissant d'herbivores mais aussi d'autres prédateurs. Les expérimentations répliquées au niveau de la communauté font défaut. Il est donc important de travailler avec un système expérimental capable de réunir la théorie et les preuves empiriques afin d'obtenir une compréhension prédictive de l'effet des assemblages de prédateurs sur la dynamique des herbivores. Des études explorant la dynamique des communautés sur plusieurs générations sont également nécessaires pour comprendre les conditions conduisant à un biocontrôle durable et à la persistance sur le long terme des ennemis naturels. Peu d'études ont également travaillé dans des conditions semi-naturelles pour évaluer comment les ennemis lâchés en masse modifient les réseaux trophiques préexistants. Le projet EnemyCocktail visera à combler certaines de ces lacunes par le biais d'une méta-analyse, de la réalisation d'expériences à différentes échelles spatiales (d'élevages au laboratoire jusqu'aux serres commerciales) et de la modélisation théorique. L'objectif principal du projet est de répondre à des questions fondamentales clés avec un objectif appliqué précis. Au niveau fondamental, nous aborderons des questions sur l'impact des ennemis naturels sur la dynamique des herbivores et comment ces interactions conduisent à la persistance des espèces ou modulent leurs extinctions. Au niveau appliqué, notre but final est de concevoir des cocktails d'ennemis naturels et de les tester dans des conditions d'élevage.

CONCLUSION GÉNÉRALE

Comme je le présente dans ce manuscrit, j'ai développé un programme de recherche très diversifié. Au niveau du modèle d'étude, j'ai commencé par les papillons de nuit, puis les pucerons et enfin les thrips. Ne pas être fidèle à un modèle a ses avantages, ce que l'on apprend d'un système peut être nouveau dans un autre et construire des ponts entre les modèles peut permettre de proposer des principes généraux. Ces avantages ont toutefois une contrepartie, car l'apprentissage et la gestion de nouveaux modèles nécessitent du temps, de l'énergie et de nombreux échecs. Même si j'ai changé plusieurs fois de système modèle, je suis resté fidèle au domaine de l'entomologie à travers le prisme de l'écologie expérimentale des communautés. Le domaine de l'écologie des communautés est vaste et je me concentrerai dans cette dernière partie du manuscrit d'un point de vue appliqué, mes intérêts peuvent être englobés dans le domaine du biocontrôle. D'une manière plus générale, je m'intéresse à la compréhension des écosystèmes terrestres dans le but ultime d'anticiper et de prévenir les conséquences dramatiques de l'impact de l'homme sur ces écosystèmes. Je discuterai également de mes perspectives de recherche futures et je présenterai mon point de vue personnel sur le mentorat des scientifiques en début de carrière. Enfin, j'évoquerai mon point de vue sur la publication en libre accès et sur les préjugés dans le milieu de la recherche.

J'ai effectué la plupart de mes recherches en étudiant le biocontrôle et la dynamique des communautés d'insectes dans des cages de population et dans des environnements de terrain simplifiés. Comme je l'ai déjà dit, ces types d'expériences ont été essentiels pour faire progresser notre compréhension des interactions entre les espèces. Toutefois, ces méthodes comportent des limites que je vais décrire ci-après, en proposant des solutions possibles. (i) La dispersion et la structure des métacommunautés sont ignorées, or nous savons que ce qui se passe dans une communauté n'est pas indépendant de ce qui se passe dans d'autres communautés connectées par la migration. (ii) La façon dont nous créons les communautés consiste à inclure un ensemble initial d'organismes que nous suivons dans le temps en ajoutant simplement de nouvelles plantes bien fertilisées chaque semaine. (iii) Lorsque nous créons des communautés, nous les dotons d'organismes dont la diversité génétique est limitée. Nous ignorons donc la dynamique éco-évolutive dans nos expériences. Je suis conscient de ces limites et certaines de mes recherches futures visent à les surmonter.

L'une de ces approches consisterait à réaliser des expériences dans des écotrons, qui sont des installations avancées à environnement contrôlé, utilisées pour reproduire des écosystèmes expérimentaux. Des expériences spécifiques peuvent être aussi réalisées pour tester le potentiel éco-évolutif des communautés étudiées, par exemple en faisant varier la quantité de génotypes différents dans les populations initiales. Une autre bonne alternative pour réaliser des expériences avec des scénarios plus réalistes est d’augmenter la taille des cages utilisées ou de se placer en situations réelles. C'est exactement ce que nous proposons dans le projet collaboratif EnemyCocktail que nous venons de lancer.

L'un des principaux aspects appliqués de mes recherches a été de mieux comprendre les interactions écologiques complexes qui émergent lorsque des techniques de biocontrôle sont utilisées contre les ravageurs. Les avantages des techniques de biocontrôle dans l'agriculture sont évidents. On estime qu'au moins 30 % de la production agricole mondiale est perdue à cause des arthropodes ravageurs. Les insecticides chimiques sont la principale méthode de lutte contre ces organismes, même si leur utilisation soulève d'importantes préoccupations en matière de santé publique, de pollution de l'environnement et d'apparition de résistances. Le biocontrôle à base d'ennemis naturels est une solution durable pour réguler les populations de ravageurs, avec un impact limité sur la biodiversité des agrosystèmes, la santé des agriculteurs et des consommateurs tout en minimisant le risque d'émergence de résistances. Le biocontrôle apparaît donc comme un élément clé de la réussite de la transition écologique vers une production agricole intensive et durable. Des efforts importants sont déployés pour accélérer et étendre sa mise en œuvre. Par exemple, les initiatives nationales comme le plan Ecophyto en France se traduisent par une augmentation constante de l'industrie du biocontrôle au cours des dernières décennies. Ces efforts sont-ils suffisants ? J'en doute. Le plan Ecophyto, par exemple, a semblé fonctionner avec une baisse de 30 % de la production de produits phytosanitaires entre 2008 et 2021, mais ces chiffres sont repartis à la hausse l'année suivante. Je pense que les plans comme Ecophyto sont intéressants et utiles pour fixer un cap, mais une réduction réelle de l'utilisation des insecticides passera nécessairement par un changement drastique des habitudes de consommation. Pour y parvenir, une baisse des prix des produits issus de l'agriculture biologique ou intégrée est indispensable et j'espère que la recherche sur l'écologie des interactions plantes-insectes-ennemis pourra contribuer à atteindre cet objectif.

Si la situation de l'agriculture ne semble pas progresser aussi rapidement que nous le souhaiterions, celle des écosystèmes naturels n'est guère plus réjouissante. Nous vivons à une époque où tous les écosystèmes sont confrontés à des changements sans précédent liés aux activités humaines. En conséquence, une espèce sur six risque de disparaître d'ici la fin du siècle. De nombreuses études récentes font état d'un déclin marqué de l'abondance et de la diversité des insectes dans l'ensemble des écosystèmes. Pour éviter les extinctions massives, il est essentiel d'adopter une approche axée sur l’écologie des communautés. L'impact de l'homme sur les espèces est généralement transmis par le biais d'interactions avec d'autres espèces dans le réseau trophique, et la dynamique de toute espèce ne peut être comprise que si la communauté dans laquelle l'espèce est intégrée est prise en considération. Cette compréhension ne peut ignorer les nombreuses interactions complexes dont je parle dans ce document. Par exemple, certains auteurs estiment que jusqu'à 80 % des extinctions d'espèces sont indirectes (c'est-à-dire qu'elles sont déclenchées par la disparition d'autres espèces) ou dues à des effets indirects. Tout comme pour le biocontrôle, les stratégies adoptées par notre société pour atténuer la dégradation de la plupart des écosystèmes de la planète semblent insuffisantes pour prévenir la situation catastrophique qui se profile. Des initiatives militantes désespérées et légitimes comme *Extinction Rebellion* ou *Les soulèvements de la terre* sensibilisent notamment les jeunes générations. En tant qu'écologiste et en tant que père, je crois que j'ai l'obligation d'agir et vite, mais je crains que notre génération ne soit trop gâtée pour agir à la mesure qu'exige la situation.

Tout le travail que j'ai décrit ci-dessus n'aurait pas été possible sans les nombreux scientifiques et praticiens du biocontrôle avec lesquels j'ai travaillé. J'ai collaboré et supervisé de nombreux scientifiques en début de carrière, y compris des candidats à la maîtrise et au doctorat. J'ai fait de mon mieux pour être un bon mentor pour eux en assurant une transition en douceur entre le point de départ et le point d'arrivée. Pour chaque scientifique en début de carrière, ce chemin était très différent, qu'il s'agisse de scientifiques académiques qui voulaient poursuivre une carrière dans le milieu de la recherche, de ceux qui voulaient s'installer dans le secteur privé, ou encore de ceux qui n'étaient pas sûrs de ce qu'ils voulaient faire. J'ai fait partie de nombreux laboratoires et, à chaque étape, j'ai beaucoup appris sur ce qu'un bon mentor peut apporter, mais aussi sur ce qu'un bon superviseur ne doit pas faire. Faire passer les intérêts du chercheur principal (ou de toute une équipe ou d'un projet de recherche) avant ceux du scientifique en début de carrière est probablement le conflit le plus important à éviter, mais aussi le plus difficile à éviter. À cet égard, j'ai adhéré à l'initiative #MentorFirst lancée par Jennifer M. Heemstra et Neil K. Garg [1]. L'initiative stipule que "En tant que professeurs de sciences, nous sommes censés produire une recherche scientifique de haute qualité. Bien qu'il s'agisse d'un objectif central, il est atteint en dirigeant des équipes d'étudiants, de postdocs et de personnel de recherche. Ainsi, notre succès dans la production de recherche est inextricablement lié au rôle important que nous jouons en tant que mentors pour les chercheurs de nos laboratoires. Nous pensons que le fait de donner la priorité aux besoins des chercheurs et de se concentrer sur la fourniture d'un mentorat de haute qualité conduit à une plus grande créativité et, en fin de compte, à une plus grande productivité dans nos programmes de recherche".

Une part importante de notre recherche consiste à mettre nos résultats à la disposition de la communauté scientifique. Pour ce faire, nous publions dans des revues à comité de lecture. Ces dernières années ont vu l'initiation de ce qui pourrait devenir un changement de paradigme en matière de publications scientifiques. La publication Open Access et l'accès public aux données sont de plus en plus demandés par les agences de financement et les institutions publiques telles que l'Union européenne, conformément aux principes FAIR de la trouvabilité, de l'accessibilité, de l'interopérabilité et de la réutilisation des données. De nombreuses voix s'élèvent également contre la marchandisation du travail des scientifiques par des éditeurs à but lucratif. Par exemple, DAFNEE, une base de données de revues universitaires en écologie et en évolution [2], répertorie 398 revues à but non lucratif, sociétés savantes ou associées à des universités dans le domaine de l'écologie et de la biologie évolutive. D'autres stratégies encore plus attrayantes se développent également. Je suis particulièrement enthousiasmé par l'initiative Peer Community In (PCI) [3], un "processus de recommandation gratuit de prépublications scientifiques basé sur des évaluations par les pairs et une revue". Dans ce nouveau système, les preprints sont envoyés et évalués par des pairs, puis recommandés. Après recommandation, les articles peuvent être envoyés directement à des revues traditionnelles ou au Peer Community Journal, dont la lecture et la publication sont gratuites. Cette démarche me semble cruciale, car de nombreuses revues en libre accès

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1 https://mentorfirst.org/

2 https://dafnee.isem-evolution.fr/

3 https://peercommunityin.org/

imposent des prix qui ne sont pas abordables pour la plupart des scientifiques, en particulier dans les pays du Sud. Pour stimuler cette initiative, PCI a lancé le PCI manifesto proposant de prendre les engagements suivants : "J'accepte de soumettre au moins un de mes meilleurs articles à un PCI pour une évaluation par les pairs avant la fin de 2023 et, si cela est recommandé, de le publier dans le Peer Community Journal" ; "Je soutiens PCI et adhère à l'idée de faire du Peer Community Journal un lieu largement utilisé pour la publication d'articles de haute qualité" ; "Je ne serai lié par cette promesse que si au moins 500 autres chercheurs prennent le même engagement." Jusqu'à présent, le manifeste a été signé par plus de 1000 chercheurs, moi y compris.

Pour finir, j'aimerais donner une dimension de genre à ma recherche. Depuis la montée en puissance du mouvement #MeToo en 2017, le souhait de voir notre société changer et combattre ouvertement l'insécurité et la discrimination est devenu une réalité. Les sociétés d'étude de l'écologie et de l'évolution n'ont pas fait exception. Avoir des événements LGBT dans les conférences, par exemple, est en train de devenir la norme plutôt que l'exception. Ces initiatives ont eu un impact important sur la manière dont je conçois actuellement la recherche. J'ai passé mon doctorat sous la supervision de trois hommes et les deux collaborateurs principaux de mes postdocs étaient également des hommes. Les six premiers articles que j'ai publiés ont été rédigés exclusivement par des hommes. Est-ce un hasard ? Je ne le crois pas. Nous sommes extrêmement biaisés et le fait de s'en rendre compte peut nous aider à prendre du recul et à surmonter ces préjugés. Ma liste de coauteurs est encore très dominée par des hommes, mais de plus en plus de femmes sont incluses dans mes projets, et par conséquent co-autrices de mes manuscrits. Il existe de nombreuses manières d'atteindre la parité, et je mets de plus en plus ces stratégies en pratique. Un moyen facile d'atteindre la parité dans les équipes ou les projets de recherche consiste à tenir compte de nos préjugés lors des recrutements en mettant en place des comités de sélection mixtes. Il s'agit d'une procédure courante dans tous les recrutements que je réalise.

Depuis le début de mon doctorat, j'ai assisté à une évolution spectaculaire de nombreux aspects du monde universitaire et de la recherche sur les insectes. La publication en libre accès devient une réalité, nous sommes plus conscients de la discrimination et de nombreuses expériences montrent qu'il est possible de cultiver sans pesticides. Nous sommes encore loin d'avoir atteint tous ces objectifs et j'espère que mon programme de recherche y contribuera. Ce programme implique un solide programme de recherche sur l'écologie expérimentale qui va de pair avec un fort investissement dans la garantie d'un milieu de recherche bienveillant pour les scientifiques jeunes et pour les générations à venir.

PART I: CURRICULUM VITAE

CURRICULUM VITAE

Enric Frago

Researcher at CIRAD - UMR CBGP

Centre de Coopération Internationale en Recherche Agronomique pour le Développement - Centre de Biologie pour la Gestion des Populations

Campus international de Baillarguet, 755 avenue du Campus Agropolis CS 30016, 34988 Montferrier sur Lez Cedex, France

e-mail: enric.frago@cirad.fr. Tel. +33 (0) 664464604

ORCID iD: 0000-0001-8817-1303

Twitter: @EnricFrago

Personal web page: https://sites.google.com/site/enricfrago/

Languages: French and English (advanced level), Italian (basic spoken), Catalan and Spanish (native)

EDUCATION

PhD in insect ecology, July 2008. Population dynamics of *Euproctis chrysorrhoea* L. (Lymantriidae) feeding on *Arbutus unedo* L. (Ericaceae) and its associated parasitoids in four Mediterranean forests. Advised by Jesús Selfa, Juli Pujade-Villar and Miguel Guara. Universitat de València (València, Spain). *Eximia cum Laude Approbatur.*

MSc in Animal and Marine Biology, June 2005. Life history of the browntail moth *Euproctis chrysorrhoea* L. (Lymantriidae) on the strawberry tree *Arbutus unedo* L. (Ericaceae). Universitat de València (València, Spain).

BSc Biology, June 2002. Universitat de València (València, Spain).

EMPLOYMENT

2019-: Researcher at CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), UMR CBGP - Centre de Biologie pour la Gestion des Populations (Montferrier sur Lez Cedex, France).

2016-19: Researcher at CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), UMR PVBMT: Peuplements Végétaux et Bioagresseurs en Milieu Tropical (Saint-Pierre, La Réunion, France).

2013-15: Marie Curie Intra-European Fellowship with Marcel Dicke at Laboratory of Entomology, Wageningen University (Wageningen, the Netherlands).

2013-15: Research Associate at Department of Zoology, University of Oxford (Oxford, UK).

2010-12: Post-doctoral fellow with Charles Godfray at Department of Zoology,University of Oxford (Oxford, UK).

2008-10: Research Associate at Departament de Zoologia, Universitat de València (València, Spain).

PUBLICATIONS

* denotes corresponding author

(Review) denotes the manuscript is a review or a meta-analysis

In bold early career scientists I have collaborated with

2023

1. E Frago*, SE Zytynska (In press) Impact of herbivore symbionts on parasitoid foraging behaviour. **Current Opinion in Insect Science**, 101027. (Review)

2022

2. E Frago*, R Gols, R Schweiger, C Müller, M Dicke, HCJ Godfray (2022) Herbivore-induced plant volatiles, not natural enemies, mediate a positive indirect interaction between insect herbivores. **Oecologia** 2: 1-14.

2021

3. B Facon*, A Hafsi, M Charlery De La Masseliere, S Robin, F Massol, M Dubart, J Chiquet, E Frago, F Chiroleu, P-F Duyck, V Ravigné (2021) Joint species distributions reveal the combined effects of host plants, abiotic factors and species competition as drivers of species abundances in fruit flies. **Ecology Letters** 24 (9): 1905-1916.
4. **R Kehoe**, E Frago, D Sanders* (2021) Cascading extinctions as a hidden driver of insect decline. **Ecological Entomology** 46 (4): 743-756. (Review)
5. SE Zytynska*, **K Tighiouart**, E Frago (2021) Benefits and costs of hosting facultative symbionts in plant-sucking insects: A meta-analysis. **Molecular Ecology** 30 (11): 2483-2494. (Review)
6. D Sanders, E Frago, **R Kehoe**, C. Patterson, K.J. Gaston* (2021) A meta-analysis of biological impacts of artificial light at night. **Nature Ecology & Evolution** 5 (1): 74-81. (Review)

2020

7. A Goldarazena*, M Matsumoto, T Ranarilalatiana, **NT Dianzinga**, E Frago, B Michel (2020) *Dendrothripoides moundi* (Thysanoptera, Thripidae), a new species from Madagascar. **Zootaxa** 4877 (2): 396-400.
8. **NT Dianzinga**, M-L Moutoussamy, J Sadeyen, LHR Ravaomanarivo, E Frago* (2020) The interacting effect of habitat amount, habitat diversity and fragmentation on insect diversity along elevational gradients. **Journal of Biogeography** 47 (11): 2377-2391. *Journal Cover Image.*
9. E Frago*, SE Zytynska, NE Fatouros (2020) Microbial symbionts of herbivorous species across the insect tree. **In: Mechanisms underlying microbial symbiosis** (Ed. JA Russell, KM Oliver, Academic Press). Chapter 4: 111-160. (Review)
10. **R Marrao**, E Frago, JA Pereira, A Tena* (2020) An autoparasitoid wasp, inferior at resource exploitation, outcompetes primary parasitoids by using competitor females to produce males. **Ecological Entomology** 45(3): 727-740.
11. A Goldarazena*, **NT Dianzinga**, E Frago, B Michel, P Reynaud (2020) A new species of the genus Thrips (Thysanoptera, Thripidae) from Malagazy region. **Zootaxa** 4750 (3): 443-446.

2019

12. E Frago*, H-L Wang, GP Svensson, **JF Marques**, JA Hódar, GH Boettner, C Ciornei, L Dormont, JS Elkinton, M Franzén, A Khrimian, L Marianelli, L Marziali, H Mas, E Perez Laorga, J Pérez-López, A Roques, V Simonca, O Anderbrant (2019) Common pheromone use among host-associated populations of the browntail moth, *Euproctis chrysorrhoea*, displaying different adult phenologies. **Entomologia Generalis** 39(3-4): 295-306.
13. A Mennerat, E Frago (2019) The response of interacting species to biotic seasonal cues. **Peer Community in Ecology** 1, 100022.
14. **LS Monticelli**, Y Outreman, E Frago, N Desneux* (2019) Impact of host endosymbionts on parasitoid host range–From mechanisms to communities. **Current Opinion in Insect Science** 32: 77-82. (Review)

2018

15. JC Streito*, O Fontaine, M Atiama, G Genson, E Pierre, J Sadeyen, E Frago (2018) Présence sur l'île de La Réunion de deux espèces de Punaises prédatrices potentiellement utilisables pour la lutte biologique: *Orius naivashae* et *Cyrtopeltis callosus.* **Bulletin de la Société Entomologique de France** 123(1): 29-42.
16. **LR Paniagua-Voirol**, E Frago, M Kaltenpoth, M Hilker, NE Fatouros* (2018)

Bacterial symbionts in Lepidoptera: their diversity, transmission, and impact on the host. **Frontiers in Microbiology** 9: 556. (Review)

2017

17. E Frago*, **M Mala,** BT Weldegergis, **C Yang**, A McLean, HCJ Godfray, R Gols, M Dicke (2017) Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles. **Nature Communications** 8: 1860.
18. **SJJ Schreven**, E Frago, A Stens, PW de Jong, JJA van Loon* (2017) Contrasting effects of heat pulses on different trophic levels, an experiment with a herbivore-parasitoid model system. **PLoS ONE** 12(4) e0176704.
19. **NH Davila Olivas**, E Frago, MPM Thoen, KJ Kloth, FFM Becker, JJA van Loon, G Gort, JJB Keurentjes, J van Heerwaarden, M Dicke* (2017) Natural variation in life-history strategy of Arabidopsis thaliana determines stress responses to drought and insects of different feeding guilds. **Molecular Ecology** 26: 2959–2977.
20. **L Fontana-Bria**, J Selfa, C Tur, E Frago* (2017) Early exposure to predation risk carries over metamorphosis in two distantly related freshwater insects. **Ecological Entomology** 42(3): 255–262. *Editor's choice within the 42:3 issue.*
21. E Frago, B Facon (2017) New partner at the core of macrolichen diversity. **Peer Community in Evolutionary Biology** 100009.

2016

22. D Sanders, **R Kehoe**, FJF van Veen, A McLean, HCJ Godfray, M Dicke, R Gols, E. Frago* (2016) Defensive insect symbiont leads to cascading extinctions and community collapse. **Ecology Letters** 19: 789–799.
23. **R Kehoe**, E Frago, CD Barten, F Jecker, FJF van Veen, D Sanders* (2016) The impact of non-host diversity and density on the strength of parasitoid-host interactions. **Ecology and Evolution** 6 (12): 4041-4049.
24. A Pekas, A Tena, JA Harvey, F Garcia-Marí, E Frago* (2016) Host size and spatiotemporal patterns mediate the coexistence of specialist parasitoids. **Ecology** 97(5): 1345–1356.
25. E Frago* (2016) Interactions between parasitoids and higher order natural enemies: intraguild predation and hyperparasitoids. **Current Opinion in Insect Science** 14: 81–86. (Review)

2015

26. **FG Pashalidou**, E Frago, **E Griese**, EH Poelman, JJA van Loon, M Dicke, NE Fatouros* (2015) Early herbivore alert matters: plant-mediated effects of egg deposition on higher trophic levels benefit plant fitness. **Ecology Letters** 18 (9): 927-936.

27. NE Fatouros*, **LR Paniagua Voirol**, F Drizou, QT Doan, A Pineda, E Frago, JJA van Loon (2015) Role of male-derived compounds in elicitation of direct and indirect egg-killing defenses in the black mustard plants. **Frontiers in Plant Science** 6:794.

2014

28. **J Lazebnik**, E Frago, M Dicke, JJA van Loon* (2014) Phytohormone mediation of interactions between herbivores and plant pathogens. **Journal of Chemical Ecology (Special Issue: Phytohormones)** 40(7): 730–741. (Review)
29. JF Marques*, H Wang, GP Svensson, E Frago, O Anderbrant (2014) Genetic divergence and evidence for sympatric host-races in the highly polyphagous Brown tail moth, *Euproctis chrysorrhoea* (Lepidoptera: Erebidae). **Evolutionary Ecology** 28(5): 829–848.
30. E de la Peña*, V Vandomme, E Frago (2014) Facultative endosymbionts of aphid populations from coastal dunes of the North Sea. **Belgian Journal of Zoology** 144(1): 41-50.
31. E Frago*, HCJ Godfray (2014) Avoidance of intraguild predation leads to a long-term positive trait-mediated indirect effect in an insect community. **Oecologia** 174(3): 943-952.
32. E Frago*, É Bauce (2014) Life-history consequences of chronic nutritional stress in an outbreaking insect defoliator. **PLoS ONE** 9 (2): e88039.

2013

33. D Giron*, E Frago, G Glevarec, C Pieterse, M Dicke (2013) Cytokinins as key regulators in plant interactions with microbes and insects. **Functional Ecology (Special Feature on Plant-Microbe-Insect Interactions)** 27 (3): 599-609. *Journal Cover Image.* (Review)

2012

34. E Frago*, M Dicke, HCJ Godfray (2012) Insect symbionts as hidden players in insect-plant interactions. **Trends in Ecology & Evolution** 27(12): 705-711. (Review)
35. E Frago*, J Pujade-Villar, M Guara, J Selfa (2012) Hyperparasitism and seasonal patterns of parasitism as potential causes of low top-down control in *Euproctis chrysorrhoea.* **Biological Control** 60(2): 123-131.

2007-11

36. E Frago*, J Pujade-Villar, M Guara, J Selfa (2011) Providing insights into browntail moth local outbreaks by combining life table data and semi-parametric statistics.

Ecological Entomology 36(2): 188-199.

37. E Frago*, M Guara, J Pujade-Villar, J Selfa (2010) Winter feeding leads to a shifted phenology in the browntail moth *Euproctis chrysorrhoea* on the evergreen strawberry tree *Arbutus unedo*. **Agricultural and Forest Entomology** 12(4): 381-388.
38. E Frago*, J Selfa, J Pujade-Villar, M Guara, É Bauce (2009) Age and size thresholds for pupation and developmental polymorphism in the browntail moth, *Euproctis chrysorrhoea* under conditions that either emulate diapause or prevent it. **Journal of Insect Physiology** 55(10): 952-958.
39. J Tormos*, E Frago, J Selfa, JD Asís, J Pujade-Villar, M Guara (2007) Description of the final instar of *Trichomalopsis peregrina* (Hymenoptera, Pteromalidae), with data and comments on the preimaginal stages. **Florida Entomologist** 90(1): 180-183.

PUBLICATIONS IN NON-REFEREED JOURNALS AND BLOG POSTS

1. E Frago (2020) Satellite images to understand the diversity of minute insects. **Journal of Biogeography highlighted papers blog posts.** [4]
2. **L Fontana-Bria**, E Frago, **E Prieto-Lillo**, J Selfa (2017) Biogeographic evaluation of the dragonflies and damselflies in the Eastern Iberian Peninsula. **Arxius de Miscel·lània Zoològica** 1, 8-29.
3. E Frago. Host plant specialization in the browntail moth, *Euproctis chrysorrhoea*, when feeding on the evergreen host Arbutus unedo. **Annales de l'Institut National de Recherche en Génie Rural, Eaux et Forêts** (Tunisia).
4. L Fontana-Bria, E Frago, J Selfa (2012) Nuevas citas de *Onychogomphus costae* Sélys, 1885 (Odonata: Gomphidae) del este de la Península Ibérica. **Boletín de la Sociedad Española de Entomología**, 50: 573-574. *In spanish.*
5. E Frago, M Guara, J Selfa, J Pujade-Villar (2011) Segona cita a la península Ibèrica d'*Elasmus nudus* (Nees, 1834)(Hymenoptera: Eulophidae), aspectes morfològics i biològics d'aquesta espècie. **Orsis**, 25: 147-155. *In catalan.*
6. E Frago, E Portuondo-Ferrer, JL Fernández-Triana*, O Sariego, J Garcés-Fonseca (2010) Entomofauna del Parque Nacional Desembarco del Granma, Cuba suroriental. **Boletín de la Sociedad Española de Entomología**, 46: 355-362. *In spanish.*

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[4] https://journalofbiogeographynews.org/2020/11/30/satellite-images-to-understand-the-diversity-of-minute-insects/

CONFERENCE ORGANISATION / SCIENTIFIC COMMITTEE

2023 Integrated control in protected crops, temperate and Mediterranean climate. **International Organisation for Biological and Integrated Control (IOBC) Meeting**, Brest (France). Member of the Scientific Committee.

2022 E Frago, Kalliope Papadopoulou. Alternative tools for plant protection - gaining insights from mechanisms in plant-arthropod-microbe interactions. **Mini-symposium at CBGP** (Centre de Biologie pour la Gestion des Populations). Montpellier (France).

2021 D Andow, D Pires, E Frago. Interactions among natural enemies and their effects on biological control. **Second International Congress of Biological Control (ICBC2)**, Davos (Switzerland). Session organiser (on-line)

2014 E Frago, A Biere. Ménage à trois: ecological consequences of intricate interactions between plants, microbes and insects. **Joint Annual Meeting of the British Ecological Society and Société Française d'Écologie**, Lille (France). Symposium organiser.

PROJECTS

2023-26 ENEMYCOCKTAIL - Designing natural enemy cocktails for a better biocontrol. **ANR - Agence Nationale de la Recherche (France)**. P.I. E Frago.

2022 Does intraguild predation challenge herbivore biocontrol? **Appel à projet interne annuel CBGP** - Centre de Biologie pour la Gestion des Populations. P.I. E Frago.

2020-22 DivSym - De la Diversité aux fonctions des Symbiontes bactériens dans deux ordres d'insectes phytophages aux modes de nutrition contrastés. Projets de recherce exploratoire, **CeMEB - Centre Méditerranéen Environnement et Biodiversité (France)**. P.I. M Jousselin, E Frago.

2019-22 INTOMED - Innovative tools to combat crop pests in the Mediterranean. **PRIMA - Partnership for Research and Innovation in the Mediterranean Area (EU)**. P.I.: Kalliope Papadopoulou.

2116-18 BSV3: Gestion agroécologique des ravageurs des cultures et gestion des plantes invasives - **Actions FEADER and FEDER (EU)**. P.I.: B Reynaud, H Delatte.

2018 Flower-visiting thrips of central Madagascar and farmers' knowledge of biological control techniques. **EPIdémiosurveillance et BIOcontrôle dans le Sud-Ouest de l'Océan Indien (Epibio-Oi)(EU)**. P.I.: H Delatte.

2014-19 COST Action FA1405: Using three-way interactions between plants, microbes and arthropods to enhance crop protection and production. European Science Foundation: **COST Action (EU)**. Management Committee and coordinator of the Short Term Scientific Missions. P.I.: A Biere.

2013-14 Insect symbionts and insect community stability. **British Ecological Society, Small Projects program (UK)**. P.I.: E Frago.

2012 Insect symbionts as hidden players in insect-plant interactions. **EPAbram Cephalosporin Fund of the Sir William Dunn School of Pathology (UK)**. P.I.: HCJ Godfray.

2011-14 Diversity and structure of the community of dragonflies and damselflies in the Xúquer river basin (València, Spain). **The Regional Ministry of Education in València (Spain)**. P.I.: J Selfa.

2011-15 Sex pheromone in browntail moth, *Euproctis chrysorrhoea* L. (Lepidoptera: Lymantriidae), host races: the basis for development of sustainable methods for monitoring and control. **Carl Trygger Foundation (Sweden)**. P.I.: O Anderbrant.

FELLOWSHIPS AND AWARDS

2014 **Uyttenboogaart-Eliasen Stichting** (the Netherlands) travel grant to attend the Keystone Conference "Mechanisms and Consequences of Invertebrate-Microbe Interactions", Tahoe City, California, USA.

2013 **Marie Curie Intra-European Fellowship** with M Dicke, Laboratory of Entomology, Wageningen University (the Netherlands). 2 years.

2013 **European Science Foundation** (Frontiers of Speciation Research). Short visit travel grant with M Dicke, Laboratory of Entomology, Wageningen University (the Netherlands). (Declined).

2012 **International University of Andalucía** (Baeza, Spain). Travel grant to attend the meeting "Plant-microbe-insect interactions: from molecular mechanisms to ecological implications".

2012 **EPAbram Cephalosporin Fund of the Sir William Dunn School of Pathology** (Oxford). Post-doctoral Fellowship with HCJ Godfray, Department of Zoology, University of Oxford (England). 9 months.

2011 **European Science Foundation** (Frontiers of Speciation Research). Short Visit Travel Grant with M Dicke, Laboratory of Entomology, Wageningen University (the Netherlands). 2 weeks.

2010 **Ministry of Education in València** (Spain). Post-doctoral Fellowship with HCJ Godfray, Department of Zoology, University of Oxford (England). 18 months.

2008 **University of València** (Spain). Grant for writing Ph.D. dissertations in Catalan.

2006 **Ministry of Education and Science** (Spain). Travel Grant with E Bauce, Laval

University (Canada). 6 months.

2003 **Ministry of Education and Science** (Spain). PhD Fellowship "FPI program" with J Selfa, Department of Zoology, University of València. 4 years.

2002 **Agency for International Cooperation** (Spain). Postgraduate fellowship "Intercampus program" with JL Fernández Triana, Universidad de Bayamo (Cuba). 2 months.

2001 **Ministry of Education and Science** (Spain). Undergraduate fellowship "Becas de Colaboración program" with J Selfa, University of València. 1 year.

INVITED SEMINARS

2010-2022

1. **University of Louvain** (Belgium). Using experimental microcosms to study the dynamics of complex arthropod communities. 2022. On-line.
2. **INRAe Unit MISTRAL**, Avignon (France). La lutte biologique contre une communauté de thrips, pucerons et tétranyques, effet de la diversité des ennemis naturels et la prédation intraguilde. 2022.
3. **Wageningen University (the Netherlands)**. Workshop: Microbial symbionts of herbivorous species across the insect tree. 2021. On-line.
4. **Wageningen Evolution & Ecology Seminar (WEES)**, Wageningen (the Netherlands). The role of natural enemy diversity and intraguild predation on herbivore dynamics 2021. On-line.
5. **The Ohio State University** (US). An experimental test on the relative role of natural enemy diversity and intraguild predation on herbivore dynamics. 2021. On-line.

2019-2020

6. **CeMEB - Centre Méditerranéen Environnement et Biodiversité**, Montpellier (France). A meta-analysis on the benefits and costs of hosting secondary endosymbionts in sap-sucking insects. 2020. On-line.

2017-2018

7. **Université d'Antananarivo, Faculté des Sciences, Département d'Entomologie**, Antananarivo (Madagascar). Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles. 2018.
8. **Université d'Antananarivo, Faculté des Sciences, Département d'Entomologie**, Antananarivo (Madagascar). Plant volatiles: consequences for apparent competition. 2018.

9. **IVIA - Instituto Valenciano de Investigaciones Agrícolas**, València (Spain). Pea aphid symbionts manipulate induced plant defences. 2017.

2015-2016

10. **Centre INRA-PACA Sophia Antipolis** (France). Aphid facultative symbiont affects induced plant defences. 2016.
11. **Atelier santé des plantes du CIRAD** (France). Chemical ecology and pest control: chemicals mediate complex interactions. 2016.
12. **Atelier santé des plantes du CIRAD** (France). The role of indirect interactions in pest control. 2016.
13. **Biobest Group, Sustainable Crop Management** (Belgium). Defensive symbionts in insects: interactions in complex communities and their implications for pest control. 2015.
14. **Station d'Ecologie Expérimentale du CNRS à Moulis** (France). Insect-symbiont interactions in complex aphid communities. 2015.
15. **Department of Chemical Ecology - Universität Bielefeld** (Germany). Insect-symbiont interactions in complex aphid communities. 2015.

2013-2014

16. **Centre INRA-PACA Sophia Antipolis** (France). Insect symbionts: a hidden trophic level in insect-plant interactions. 2014.
17. **INRA center in Avignon** (France). Insect symbiont interactions with natural enemies: from complex parasitoid communities to pest control. 2014.
18. **CNRS Séminaires d'écologie et d'évolution, Montpellier** (France). Insect symbionts: a hidden trophic level in insect-plant interactions. 2014.
19. **INRA center for Ecology and Genetics of Insects, Rennes** (France). *Hamiltonella defensa* mediates top-down and bottom-up interactions in aphids. 2014.
20. **Max Planck Institute for Chemical Ecology, Jena** (Germany). Symbiosis in the plant-insect interface. 2014.
21. **Department of Biology, Lund University** (Sweden). Insect symbionts as hidden players in insect-plant interactions. 2013.

2011-2012

22. **University of Padova, Department of Environmental Agronomy – Entomology, Padova** (Italy). Insect symbionts as hidden players in interactions between forest insects and their host trees. 2012.

23. **Department of Zoology Insect Meeting, University of Oxford** (England). Insect symbionts as hidden players in insect-plant interactions. 2012.
24. **Department of Zoology, Universitat de València, València** (Spain). Insect symbionts as key players in insect-plant interactions. 2012.
25. **European Science Foundation Exploratory Workshop on Plant-Microbe-Insect interactions: from molecular mechanisms to ecological implications, Wageningen** (the Netherlands). Effects of aphid symbionts on plant-insect interactions in a multitrophic context. 2011.
26. **Yearly Entomology Laboratory Research Exchange Meeting, Wageningen** (the Netherlands). The role of pea aphid secondary symbionts in host plant use through plant induced defences. 2011.
27. **Plant-insect interaction discussion group, Laboratory of Entomology, Wageningen University, Wageningen** (the Netherlands). Induced plant defences in the context of apparent competition in aphid communities. 2011.
28. **Department of Zoology, Universitat de València, València** (Spain). Shared natural enemies, different hosts: apparent competition and its implications for pest control. 2011.

CONFERENCE PRESENTATIONS (2007-)

2021-2022

1. K Tighiouart, E Frago. Predator effects on herbivore host switch: an eco-evolutionary experiment. Ecology and Evolution: New perspectives and societal challenges - **Joint meeting of the SFE2, GfÖ, EEF societies**. Metz (France). 2022. Talk.
2. K Tighiouart, E Frago. L'effet indirect d'un ennemi naturel sur le changement de plante hôte d'un herbivore ravageur. **Meeting of the EMBA (Ecological Management of Bioagressors in Agroecosystems) network**, Avignon (France). 2022. Invited talk.
3. E Frago, L Jackson, A Xuéreb. Mycorrhizal symbiosis effects on herbivore competition and natural enemy effectiveness. **Annual Meeting of the Entomological Society of America**, Vancouver (USA). 2022. Invited talk (on-line).
4. K Tighiouart, D Sanders, E Frago. An experimental test on the relative role of natural enemy diversity and intraguild predation on herbivore dynamics. **International Congress of Entomology - ICE2020**, Helsinki (Finland). 2022. Talk.
5. K Tighiouart, D Sanders, E Frago. An experimental test on the relative role of natural enemy diversity and intraguild predation on herbivore dynamics. **Models in Population Dynamics, Ecology and Evolution (MPDEE Meeting)**, Torino (Italy). 2022. Invited talk (on-line).
6. L Jackson, A Xuéreb, E Frago. Mycorrhizal effects on arthropods from different guilds: *Bemisia tabaci*, *Aphis gossipii*, *Tuta absoluta* and *Tetranychus urticae/evansi*. **Annual**

meeting of the PRIMA INTOMED project, Braganza (Portugal). 2021. Talk.

7. K Tighiouart, D Sanders, E Frago. An experimental test on the relative role of natural enemy diversity and intraguild predation on herbivore dynamics. **42èmes journées des Entomophagistes, Rennes (France)**. 2022. Talk.
8. K Tighiouart, D Sanders, E Frago. The effect of natural enemy diversity and intraguild predation on herbivore suppression. **Second International Congress of Biological Control (ICBC2), Davos (Switzerland)**. 2022. Talk (on-line).

2019-2020

9. K Tighiouart, D Sanders, S Nibouche, E Frago. The effect of natural enemy diversity on herbivore suppression and community stability. **British Ecological Society On-line Annual Meeting**. 2020. On-line talk.

2017-2018

10. E Frago. Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles. **First International Congress of Biological Control, Beijing (China)**. 2018. Invited talk.
11. SE Zytynska, E Frago. A meta-analysis on the protective benefits and costs of hosting secondary endosymbionts in sap-sucking insects. **COST Action FA1405 Annual Meeting, Valletta (Malta)**. 2018. Talk.
12. J-P Deguine, M Jacquot, E Frago, P Laurent, L Vanhuffel, D Vincenot, J-N Aubertot. Socio-economic impacts and extension process of conservation biological control in mango orchards in Réunion Island. **Fifth International Symposium on Biological Control of Arthropods, Langkawi (Malaysia)**. 2017. Talk
13. NT Dianzinga, M-L Moutoussamy, LHR Ravaomanarivo, S Nibouche, E Frago. Signals of plant phylogeny and plant invasions on thrips diversity along elevational gradients. **Symposium on Insect-Plant interactions, Tours (France)**. 2017. Talk
14. E Frago. Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles. **COST Action FA1405 Annual Meeting, Ljubljana (Slovenia)**. 2017. Talk.

2015-2016

15. E Frago. Using aphids to bring together chemical and community ecology. **Workshop Fundamental and Applied Research in Chemical Ecology (FARCE)-CIRAD, Newchâtel (Switzerland)**. 2016. Talk.
16. E Frago. Defensive insect symbiont leads to cascading extinctions and community collapse. **COST Action FA1405 Annual Meeting, Málaga (Spain)**. 2016. Talk.

17. E Frago, M Mala, A McLean, HCJ Godfray, M Dicke, R Gols. Bacterial symbionts mediate parasitoid attraction to plant volatiles. **UK – French joint meeting on Aphids, Paris (France)**. 2015. Talk.
18. E Frago, M Mala, A McLean, HCJ Godfray, M Dicke, R Gols. Bacterial symbionts mediate parasitoid attraction to plant volatiles. **7ième journée du Réseau « Interactions Micro-organismes-Hôtes », Montpellier (France)**. 2015. Talk.
19. E Frago. Bacterial symbionts mediate complex interactions between aphids and parasitic wasps. **4th Entomophagous Insect Conference, Málaga (Spain)**. 2015. Talk.
20. E Frago. Insect symbionts mediate interactions at the community level. **Crop-Arthropod-Microorganism interactions: from molecules to modelling, Turin (Italy)**. 2015. Talk.
21. E Frago. Insect symbionts mediate indirect interactions in aphid communities. **8th Congress of the International Symbiosis Society, Lisbon (Portugal)**. 2015. Talk.
22. E Frago, R Gols, FJF van Veen, M Dicke, HCJ Godfray, D Sanders. Insect symbionts mediate indirect interactions in aphid communities. **REID: Réseau ecologie des interactions durables, Lyon (France)**. 2015. Talk.

2013-2014

23. E Frago. Insect symbionts and insect community stability. **26th Netherlandse Entomologendag, Ede (the Netherlands)**. 2014. Talk.
24. E Frago, FJF van Veen, M Dicke, HCJ Godfray. Insect symbionts: a hidden trophic level in insect-plant interactions. **Symposium on Insect-Plant interactions, Neuchâtel (Switzerland)**. 2014. Talk.
25. E Frago, M Dicke, HCJ Godfray. Insect symbionts mediate indirect interactions between insects and their host plants. **Keystone Conference on "Mechanisms and Consequences of Invertebrate-Microbe Interactions", Tahoe City, California (USA)**. 2014. Poster.
26. E Frago, M Dicke, HCJ Godfray. Herbivore-induced plant volatiles in the context of apparent competition. **INTECOL, London (UK)**. 2013. Talk.

2011-2012

27. E Frago, M Dicke, HCJ Godfray. Pea aphid defensive symbiont interactions at the community level. **British Ecological Society Annual Meeting, Birmingham (UK)**. 2012. Talk.
28. E Frago, M Dicke, HCJ Godfray. Intimate connections between insect symbionts and plants: when is an insect symbiont also a plant pathogen? **Plant-microbe-insect interactions: from molecular mechanisms to ecological implications, Baeza (Spain)**. 2012. Talk.

29. E Frago, M Dicke, HCJ Godfray. Herbivore-induced plant volatiles in the context of apparent competition. **Royal Entomological Society Meeting - ENTO'12, Cambridge (UK)**. 2012. Talk.
30. E Frago. Evidence of host race formation in the browntail moth, *Euproctis chrysorryoea.* **Entomological Research in Mediterranean Forest Ecosystems, Hammamet (Tunisia)**. 2012. Talk.
31. E Frago, HCJ Godfray. Intraguild predation avoidance leads to a positive trait-mediated indirect effect in an experimental community. **British Ecological Society Annual Meeting, Sheffield (UK)**. 2011. Talk.
32. E Frago, HCJ Godfray. Intraguild predation avoidance behaviour in the context of apparent competition in an aphid community. **Second Entomophagous Insect Conference, Antibes (France)**. 2011. Talk.

2007-2010

33. E Frago, J Pujade-Villar, M Guara, J Selfa. Characterizing browntail moth local outbreaks in the Iberian Peninsula by combining life table data and non-linear statistics. **IX European Congress of Entomology, Budapest (Hungary)**. 2010. Talk.
34. E Frago, J Pujade-Villar, M Guara, J Selfa. From spring feeder to winter feeder: ecological implications in the browntail moth, *Euproctis chrysorrhoea* L. (Lepidoptera: Lymantriidae). **International Symposium of Entomological Research in Mediterranean Forest Ecosystems, Estoril (Portugal)**. 2008. Talk.
35. E Frago, M Guara, J Pujade-Villar, J Selfa. Population dynamics of the browntail moth, *Euproctis chrysorrhoea* (Lepidoptera: Lymantriidae), in the eastern Iberian peninsula, focusing on the role of its naturally associated parasitoids. **X European Workshop on Insect Parasitoids, Erice (Italy)**. 2007. Poster.
36. E Frago, É Bauce, C Tremblay. Nutrition-related stress carries over to spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae) progeny. **Annual meeting of the Entomological Society of Canada and the Entomological Society of Québec, Montréal (Canada)**. 2007. Poster.

MENTORING / TUTORING

PhD students

2021 **Karim Tighiouart - Université de La Réunion (France)** Effets des interactions trophiques complexes sur la structure et la stabilité des communautés d'arthropodes : implications pour le Biocontrôle (co-supervised with Samuel Nibouche). *Currently advisor at* Coopérative agricole Duransia *(France).*

2020 **Niry Dianzinga - Université de La Réunion (France)** Diversité des communautés d’arthropodes et efficacité de la lutte biologique contre les insectes ravageurs (co-supervised with Samuel Nibouche and Lala Raveloson Ravaomanarivo). *Currently* chargé de mission ECOPHYTO transfert *in the French Department Mayotte.*

2017 **Laia Fontana Bria - Universitat de Valencia, València (Spain)** Odonats del País Valencià: diversitat, factors implicats en la composició de les seues comunitats i respostes als senyals de risc durant la fase d'ou (co-supervised with Jesús Selfa). *Currently lecturer at the University of València.*

MSc students

2021 **Léo Jackson - BOOST**, Sophia Antipolis (France)

2020 **Giacomo Morassutti Vitale - ISARA**, Lyon (France)

2018 **Laura Madeline - ENSAT/UPS**, Toulouse (France) (co-supervised with PhD student Niry Dianzinga).

2017 **Karim Tighiouart - Montpellier Supagro**, Montpellier (France). *Price to the best MSc Biocontrol project by the "Académie du Biocontrôle et de la Protection Biologique Intégrée".*

2017 **Sandro Moretti - BEST Ecosystèmes Terrestres**, Université de la Réunion, Saint-Pierre (Réunion) (co-supervised with Olivier Fontaine - La Coccinelle).

2016 **Chenjiao Yang - Plant Sciences**, Wageningen University (the Netherlands).

2015 **Mukta Mala - Plant Sciences**, Wageningen University (the Netherlands). *Currently postdoc at the Univeristy of Oxford, UK.*

2014 **Luis Paniagua Voirol - Plant Sciences**, Wageningen University (the Netherlands) (co-supervised with Nina Fatouros).

BSc students

2021 **Julien Tchilinguirian - M1 Ingénierie en Écologie et Gestion de la Biodiversité (IEGB)**, Université de Montpellier (France) (co-supervised with Christine Meynard).

2020 **Chloe Walter, Xheke Leka, Mouigni Hadji, Maurice Schumacher - Tutored project, Montpellier Supagro**, Montpellier (France).

2016 **Kenan Potacsek, Vincent Fontaine, Samuel Doris - Tutored project, Institut Universitaire de Technologie (IUT)**, Saint Pierre (La Réunion) (co-supervised with Bernartd Reynaud).

2014 **Alejandro Vargas - Plant Sciences**, Wageningen University (the Netherlands).

2014 **Anouschka Roepers - Biology**, Wageningen University (the Netherlands).

2014 **Nick Huijers - Biology**, Wageningen University (the Netherlands).

2012 **Catherine Gresty - Biology**, University of Oxford (UK).

2011 **Yaiza Ontoria - Biology**, University of Oxford (UK).

2008 **María del Mar Ferrer Suay - Biology**, Universitat de València (Spain).

TEACHING EXPERIENCE

2021 Talk to Bachelor students "Parasitoids and pest control". Reus (Spain). On-line.

2021 Workshop "Microbial symbionts of herbivorous species across the insect tree". University of Wageningen, Wageningen (the Netherlands). On-line

2022 One invited lecture at the Autumn School BOOST on "Complex interactions between pests and enemies, implications for biocontrol". Sofia Antipolis (France).

2019 One invited lecture at the Autumn School BOOST on "Indirect effects and implications for biocontrol", Sofia Antipolis-Nice (France).

2014 Two invited lectures on "Insect symbiosis: basic and applied aspects". Master's degree "Molecular aspects of biological interactions", Wageningen University, (the Netherlands).

2013 Supervision of mini-projects in the course Molecular Aspects of Biointeractions, Laboratory of Entomology, Wageningen University (the Netherlands).

2012 Supervision of mini-projects in the course Molecular Aspects of Biointeractions, Laboratory of Entomology, Wageningen University (the Netherlands).

2012 Two invited lectures on "Apparent competition in insect herbivores: a case study with aphids " and "Browntail moth local outbreaks in eastern Iberian Peninsula ". Master's degree "Insect Ecology", Department of Environmental Agronomy – Entomology, University of Padova (Italy).

2012 One hour lecture in the undergraduate course "Quantitative Methods", Department of Zoology, University of Oxford (UK).

2012 24 hours as demonstrator in the undergraduate course "Quantitative Methods", Department of Zoology, University of Oxford (UK).

2012 Tutorials: Worcester College (Oxford) "Short introduction to the Statistical Software JMP" (2x); Deptartment of Zoology, University of Oxford "Ecology of Insect Parasitoids" (UK).

2011-12 48 hours as demonstrator in the undergraduate course "Quantitative Methods", Department of Zoology, University of Oxford (UK).

2012 Demonstrator at the University of Oxford's UNIQ Summer School, University of Oxford (UK).

2010 Two invited lectures on "Ecology and Evolution of Insects". Master's degree "Biodiversity: Conservation and Evolution", University of València (Spain).

2007-08 60h as lecturer of the "Ethology Laboratory" class, Bachelor of Sciences degree, University of València (Spain).

2006-07 60h as lecturer of the "Ethology Laboratory" class, Bachelor of Sciences degree, University of València (Spain).

COURSES

2022 Modelling in population dynamics. INRAe - National Research Institute for Agriculture, Food and the Environment (Sophia Antipolis, France). 1 week.

2019 At the border between ecology and evolution. CeMEB - Centre Méditerranéen Environnement et Biodiversité (Montpellier, France). 1 day.

2014 Bioinformatics – a User's Approach. The graduate school Experimental Plant Sciences, Wageningen University (Wageningen, The Netherlands). 1 week.

2010 Semiochemicals in pest control and conservation biology. Swedish University of Agricultural Sciences and Lund University (Lund, Sweden). 2 weeks.

COLLECTIVE TASKS

CBGP (Centre de Biologie pour la Gestion des Populations) - Montpellier, France

- Scientific coordinator of the SEPA platform (*Serres, élevage et phénotypage d'arthropodes* - Greenhouses, rearing and phenotyping of arthropods) (2022-current)
- Member of *Conseil d'Unité* (2021-2022)
- Member of *Conseil scientifique* (2021-current)

PVBMT (Peuplements Végétaux et Bioagresseurs en Milieu Tropical) - Saint-Pierre, La Réunion

- Member of TAS 3P (*Team d'Animation Scientifique*) in charge of organising weekly seminars (2017- 2018)
- Member of the reference group for the Entomology Laboratory (2017- 2018)

Associate Editor for Entomologia Generalis 2018 - 2022.

Work as reviewer:

- 2023 (x4), 2022 (x11), 2021 (x9), 2020 (x14), 2019 (x8), 2018(x19), 2017(x14), 2016(x11), 2015(x12), 2014(x6), 2013(x3), 2012(x4), 2011(x4), 2009(x1)

- **Journals:** Acarologia, African Entomologist, Animnal Behaviour, Animal microbiome, Biological Control, Biology Letters, Bulletin of Insectology, BMC Ecology and Evolution, Communications Biology, Current Biology, Current Zoology, Ecography, Ecological Entomology, Ecology, Ecology and Evolution, Ecology Letters, eLife, Entomologia Experimentalis et Applicata, Entomologia Generalis, Evolutionary Ecology, Environmental Microbiome, Experimental and Applied Acarology, FEMS Microbiology Ecology, Frontiers in Microbiology, Frontiers in Plant Science, Functional Ecology, Heredity, Insect Science, International Journal of Insect Science, International Journal of Molecular Sciences, iScience, Isme Journal, Journal of Animal Ecology, Journal of Forest Research, Journal of Insect Behaviour, Journal of Pest Science, Microbiome, Molecular Ecology, Nature Communications, Nature Ecology & Evolution, Neotropical Entomology, Oecologia, Oikos, PeerJ, Pest Management Science, Phytoparasitica, Plant Biology, Plant Cell and Environement, Plos ONE, Population Ecology, Proceeding of the Royal Society B, Scientific Reports, Urban Forestry & Urban Greening.

Expert for funding agencies (years)

- **Agence Nationale de la Recherche**, ANR, France (2020, 2019, 2015).
- **Deutsche Forschungsgemeinschaft** - German Research Foundation (2022).
- **Fondation pour la Biodiversité - FRB-CESAB**, Montpellier, France (2021)
- **Max Planck Society**, Germany (2021).
- **Czech Science Foundation** (2019)
- **Estonian Research Council** (2016).

Juries, evaluation committees and recruiting committees (years)

- **PhD monitoring committee** of Alexandre Bout, Université Côte d'Azur, Nice, France (2021-current).
- **PhD monitoring committee** of Jess Rouil, CBGP, Montpellier, France (2021-22).
- **Recruiting committee** of a permanent post at the Plant Health Institute of Montpellier - PHIM, Montpellier, France (2021).
- ***Comité de pilotage*** project Cemeb - Bioinspiration et Solutions fondées sur la Nature (2020).
- **PhD jury** Estelle Postic, Agrocampus Ouest, Rennes, France (2020).
- **PhD jury** Eva Thomine, Université Côte d'Azur, Nice, France (2019).
- **MSc jury** Celine Poirier, Montpellier Supagro, Montpellier, France (2019).
- **PhD jury** Ruth Cebolla, Universitat de València, Valèncai, España (2018).
- **PhD jury** Lucía Torres Muros, Universidad de Granada, España (2015).

MEMBERSHIP

- Amnesty International (2017-current)
- Doctors Without Borders (2017-current)
- The International Symbiosis Society (2015-current)
- Société Française d'Écologie (2015-current)
- The Royal Entomological Society (2014-current)
- The Spanish Society of Terrestrial Ecology (2013-current)
- British Ecological Society (2011-current)

OUTREACH

2022 Talk "La lutte biologique contre une communauté de thrips, pucerons et tétranyques, effet de la diversité des ennemis naturels et la prédation intraguilde". Association Jardin Nature Pibrac (France).

2022 Talk "Le financement dans un "Établissement public à caractère industriel et commercial" (EPIC), quel est le prix à payer pour la recherche ?" Journée thématique SFE^2 2022 « Éthique du Financement de la recherche en écologie et évolution »

2018 Group discussion about "Tomato production in Reunion and associated pests" at the UMT SPAT (*Unité Mixte Technologique Santé végétale et production agro-écologique en milieu tropical* - Mixed Technical Unit on Plant health and agroecological production in tropical habitats), Saint-Pierre (La Réunion).

2017 Group discussion about "Integrated pest control: natural enemies" at Cultur'Agri organised by the Biocontrol company *La Coccinelle*, Saint-Pierre (La Réunion).

2017 H Delatte, E Frago. Comprendre les insectes : les espèces et les interactions. Les rencontres AgroFert'iles: Journées professionnelles à l'Armeflhor, Saint-Pierre (La Réunion).

2017 Invited talk about "Thrips diversity and management in Reunion greenhouses" at the UMT SPAT (*Unité Mixte Technologique Santé végétale et production agro-écologique en milieu tropical* - Mixed Technical Unit on Plant health and agroecological production in tropical habitats), Saint-Pierre (La Réunion).

RESEARCH STAYS

2015 **University of Exeter** (UK) with Dirk Sanders. 1 month.

2013 **University of Padova**, DAFNAE (Italy) with Lorenzo Marini. 2 months.

2006 **Laval University** (Canada) with E Bauce. 6 months.

2002 **Universidad de Bayamo** (Cuba) with JL Fernández Triana. 2 months.

CURRENT COLLABORATIONS

(in alphabetical order)

CBGP (Centre de Biologie pour la Gestion des Populations) - Montpellier, France.

Philippe Auger, Laure Benoit, Anne-Laure Clamens, Emmanuelle Jousselin, Anne Loiseau, Christine Meynard, Bruno Michel, Denise Navia, Sabine Nidelet, Diego Santos García, Marie-Stéphane Tixier, Anne Xuéreb.

Biobest Group - Belgium / France

Apostolos Pekas and Azélie Lelong

IVIA (Valencian Institute for Agricultural Research) - València, Spain.

Alejandro Tena

PRIMA INTOMED project (Innovative tools to combat crop pests in the Mediterranean).

Kalliope Papadopoulou (University of Thessaly, Greece), Maria Pappas and George Broufas (Democritus University of Thrace, Greece), Dimitrios Arrianas (Entreprise FYTOTHREPTIKI, Greece), Victor Flors and Paloma Sánchez Bel (Jaume I University, Spain), Rachid Mentag (National Institute of Agricultural Research of Morocco, Morocco), Paula Baptista (Instituto Politécnico de Bragança, Portugal), Meriem M'saad Guerfali (Centre National des Sciences et Technologies Nucléaires, Tunis), Salma Ben Romdhane (Centre Technique des agrumes, Tunis).

The Herbivory Variability Network

William Wetzel (Michigan State University, USA), Moria L. Robinson (Utah State University, USA)

University of Exeter, Environment and Sustainability Institute, Exeter, UK

Rachel Kehoe and Dirk Sanders

University of Liverpool, Department of Ecology, Evolution and Behaviour, UK

Sharon Zytynska

University of Oxford, Department of Zoology

Ailsa McLean and Mukta Mala

University of Wageningen, Biosystematics

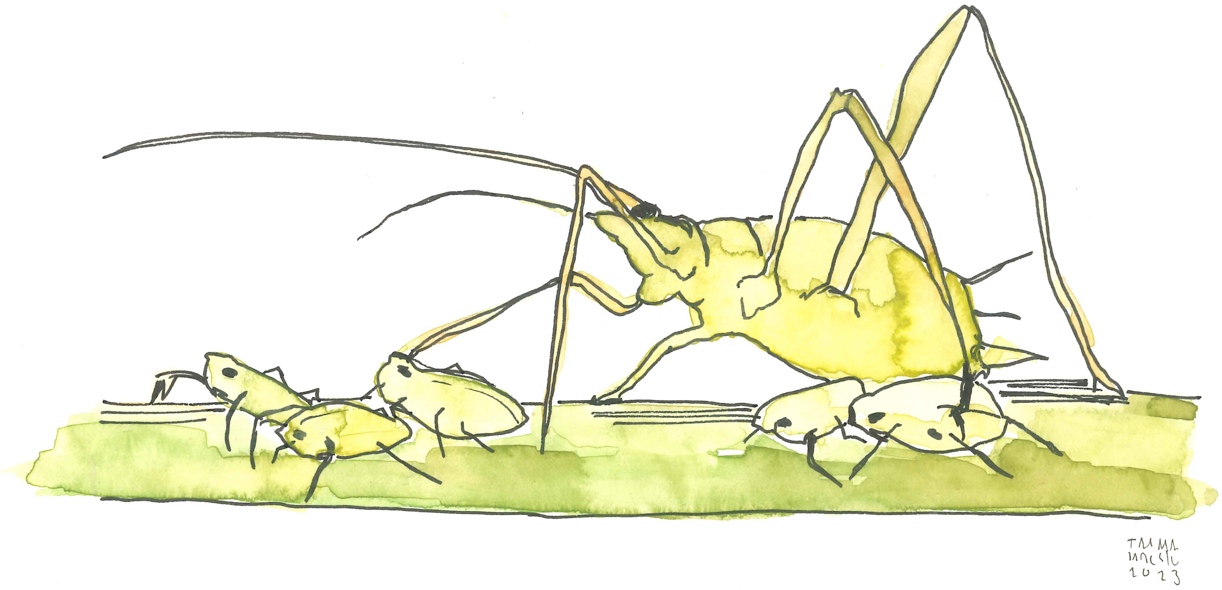
Nina Fatouros

PART II. SCIENTIFIC MEMOIR

GENERAL CONTEXT

Insect herbivores are at the base of some of the most complex communities on Earth, and understanding their dynamics has long interested ecologists. This interest lies on the pure curiosity to understand nature, but also on the need to manage agricultural and forest pests. Insect herbivores are located "between the devil and the deep blue sea" because they have to face anti-herbivore plant defences, while avoiding or resisting natural enemies (Lawton & McNeill, 1979). These two forces, also expressed as top-down (i.e. enemy effects on herbivores) and bottom-up (i.e. plant effects on herbivores), act simultaneously, and the debate on how relatively important they are in determining herbivore dynamics is as old as unresolved. Herbivore population dynamics can be therefore driven by plant defences, natural enemies, abiotic variables or by their combinations. The dominating element, however, is species and system specific, and even within the same system the balance between these forces is highly variable through time and space (Morin, 2011). If these intricate interactions make the understanding of herbivore dynamics difficult, the picture has become even more complicated with the recent realisation of the importance of symbionts for insect biology. This realisation has been possible thanks to technological advances, and molecular biology in particular, which have made the study of microbes more open and accessible to non-model organisms. Insect symbionts are able to facilitate herbivore feeding on plants, but also to protect their hosts from natural enemies, so they are now considered as an integral part of herbivore trophic webs (McLean et al., 2016). In this section I will discuss my past and present research, and the sections will be structured by taking a central focus on herbivores, and on how their dynamics are determined by plant defences, natural enemies and their symbionts.

Chapter 1: Plant-mediated interactions between insect herbivores



The pea aphid *Acyrthosiphon pisum* feeding on a Fabaceae plant
(watercolour on paper by tramabarcelo)

1.1. CONTEXT

The first studies on bottom-up effects of plants on herbivores already identified that in addition to cellulose and lignin, plants marinate these two basic elements with a complex cocktail of metabolites, known as plant secondary metabolites (Iason et al., 2012). Initially considered as waste products of plant metabolism, Fraenkel (1959) was the first author to identify them as defences resulting from the evolutionary arms race against herbivores. In a classic paper, Feeny (1970) showed that fitness and population growth of the winter moth *Operophtera brumata* may depend on leaf toughness, but also on the concentration of proteins, sugar and more importantly, leaf tannins. Tannins are phenolic compounds that are at low concentration at the beginning of the season when oak leaves burst, and their concentration increases as the season progresses. These changes make leaves unpalatable, and this is why caterpillars must hatch in synchrony with bud-burst. Even a few days of asynchrony between egg hatch and bud-burst can have dramatic consequences for caterpillars. During my PhD I spent six months in Quebec, and in collaboration with Éric Bauce we studied adaptation to these types of chemical defences in an important forest moth, the spruce budworm, *Choristoneura fumiferana.* As with the winter moth *O. brumata* mentioned above, *C. fumiferana* fitness decreases sharply when post-overwintering larvae feed on old foliage of balsam fir, *Abies balsamea* (Carisey & Bauce, 1997a, 1997b, 2002; Fuentealba et al., 2017). Old foliage is tougher (Fuentealba et al., 2020), it has lower concentrations of basic nutritional elements and increased concentrations of defensive chemicals like phenols (Delvas et al., 2011). We fed larvae on artificial diets emulating fresh vs old foliage during several generations. We observed that chronic nutritional stress dramatically reduced fitness and triggered changes in life-history traits (Frago & Bauce, 2014). *C. fumiferana* is a species that outbreaks cyclically, and during outbreaks large densities of larvae may force these populations to switch from feeding on new to old foliage. Our study thus suggested that density-dependent deterioration in plant quality may be an important driver of outbreak declines in this species.

Tannins and all the diversity of secondary compounds found in plants are now recognised to modulate interactions, not only with herbivores, but also with mutualists like pollinators, plant competitors and abiotic stressors (Bennett & Wallsgrove, 1994; Iason et al., 2012). The production of these compounds, however, is metabolically expensive, and to save costs in the absence of herbivores, plants have evolved induced defences (Schoonhoven et al., 2005). Similar to the immune system of animals, induced defences are only triggered upon herbivore or pathogen attack, and they are highly specific to tailor resistance to the particular attacker triggering the response. The defences act upon detection of specific types of feeding damage and/or compounds in herbivore's oral secretions (Erb et al., 2012; Erb & Reymond, 2019). One way plants fine-tune such defences is via phytohormones, which are at the base of the signal-transduction pathways leading to the expression of defence genes (Erb et al., 2012; Stam et al., 2013). There are several phytohormones involved in induced plant defences, but the most well studied are jasmonic acid, salicylic acid and ethylene. In addition to these three, plant responses to insect attack, or to environmental stressors are dependent on other less studied phytohormones including auxins, gibberellins, cytokinins, and brassinosteroids (Bari & Jones, 2009; Pieterse et al., 2012; Giron et al., 2013; Lazebnik et al., 2014). Ethylene, jasmonic and salicylic acid are three important hormones in plants because they are key regulators of plant responses to herbivory. The expression of these phytohormones depends on the feeding guild of the herbivore. While plant responses to leaf-chewing herbivores are mainly regulated by jasmonic acid and ethylene, responses to sap-sucking herbivores mostly induce the salicylic acid pathway. Since plant resources to induce a response are limited, different pathways interact through a resource allocation trade-off (or crosstalk): upregulation of one pathway downregulates the other (Bari & Jones, 2009; Pieterse et al., 2012; Thaler et al., 2012a; Lazebnik et al., 2014; Erb & Reymond, 2019). Such resource allocation trade-off has been centred on jasmonic and salicylic acid, and to a lesser extent on ethylene. The jasmonic acid - salicylic acid crosstalk has long been considered crucial to understand the dynamics of herbivores on plants. When plants experience herbivory by multiple species, specific responses to counter a given species can modulate plant resistance to another. Herbivores sharing plants thus interact indirectly through changes in plant physiology.

The induced plant defences I discussed so far imply changes in the concentration and toxic potential of some secondary metabolites in leaves. Other plant defences that are induced by herbivory include changes in plant physical defences like glandular trichomes, leaf pubescence, and waxes that may reduce herbivory (Schoonhoven et al., 2005). Plant defences also involve the emission of plant volatiles known as herbivore-induced plant volatiles (often abbreviated as HIPVs). Upon herbivore attack, these organic volatile compounds are created *de novo* or if constitutively present they increase in concentration. Herbivore induced plant volatiles can enhance plant fitness directly if they reduce feeding rate of herbivores, or if they repel them. For example, resistance of Pedunculate oaks *Quercus robur* to the pest moth *Tortrix viridana* can be enhanced by selecting tree genotypes that emit repelling volatiles like the sesquiterpenes α-farnesene and germacrene-D (Ghirardo et al., 2012). Plant volatiles can also act as indirect defences if they attract herbivore's natural enemies (De Moraes et al., 1998; Dicke & Baldwin, 2010; Pieterse et al., 2013). In this interaction type the indirect effect occurs between the herbivore and its natural enemy through changes in the plant. Such a line of defence has attracted a lot of attention during the last 30 years. Induced volatiles have attracted interest of ecologists, but also of applied entomologists because plants can be selected or engineered to better attract pest enemies (Turlings & Erb, 2018). The attractive role of herbivore-induced plant volatiles was initially discovered almost in parallel in predatory mites (Dicke & Sabelis, 1987) and parasitoid wasps (Turlings et al., 1990). In both cases, relative to clean plants, volatiles emanating from plants infested with spider-mites *Tetranychus urticae* or by beet armyworm larvae *Spodoptera exigua* were more attractive, respectively, to the predatory mite *Phytoseiulus persimilis* and the parasitic wasp *Cotesia marginiventris*. As I will detail in the following section, my main interest in plant defences has been on their capability of modulating interactions between species, and therefore to structure herbivore communities. More precisely, changes in plant physiology brought by herbivory will have consequences for other species thriving on this same plant (Bukovinszky et al., 2008; Xiao et al., 2012; Hilker & Fatouros, 2015; Poelman & Kessler, 2016). If volatiles are involved, interactions can even occur between herbivores feeding on different plants.

1.2. PLANT DEFENCES STRUCTURE HERBIVORE COMMUNITIES

a. Plant phytohormones determine herbivore interactions

Early research on plant defences was dedicated to understanding the molecular and physiological bases of the expression of defence genes, and subsequent synthesis of metabolites (Schoonhoven et al., 2005). More recently, there has been an increasing interest on how these defences influence herbivore communities. Constitutive chemicals, for example, can greatly impact the community of herbivores associated with a given plant. In a field experiment, Bukovinszky et al. (2008) exposed two different varieties of cabbage plants *Brassica oleracea* to the natural community of herbivores and their natural enemies. The authors found that the two varieties were colonised by very different trophic webs, with differences cascading up to the third trophic level thus spanning herbivores, parasitoids and hyperparasitoids. The structure of herbivore communities can also depend on induced plant defences. Feeding by herbivores triggers plastic changes in plants with consequences for other insects feeding on this same plant, a plant-mediated indirect effect. The ubiquity of this type of indirect interaction has long been recognised, but appreciation of community-wide consequences are more recent (Van Sandt & Agrawal, 2004; Ohgushi, 2005; Viswanathan et al., 2005; Ohgushi, 2008; Poelman et al., 2008b). One of the earlier studies showing the dramatic consequences that early herbivory has on the succession of herbivores on plants was the study by Poelman et al. (2008a). In a field experiment, the authors of this work exposed cabbage plants *Brassica oleracea* to the natural community of herbivores and enemies. At the beginning of the experiment, however, defences of some of these plants were induced through herbivory by the smallwhite butterfly *Pieris rapae.* This butterfly is a chewing herbivore that munches on leaves, and as expected, plants that suffered early herbivory overexpressed the LOX2 gene, which plays a key role in the biosynthesis of jasmonic acid. These changes reprogrammed the plant metabolism and over the course of thirty-five weeks the abundance of different lepidopterans colonising these plants was also altered. Surprisingly, the density of *P. rapae* butterflies (the one that induced plants at the beginning) was unaltered, but induction increased densities of *Plutella xylostella*, while reducing those of *Mamestra brassicae.* This and the many other similar studies listed before showed that early herbivory modulates the seasonal succession of species that a plant will ultimately be fed upon, and were the embryo of a novel research field. Competition between herbivores moved from pure competition for the plant resource to subtle interactions modulated by intricate cascades of phytohormones that regulate the expression of defence-related genes. For example, as with resource competition, we know now that in plant-mediated indirect effects some species may play more important roles than others. These species may have the greatest impact on the full community of species attacking a plant, and ultimately on plant fitness. The other species simply occupy the niche left by dominant species after the reconfiguration of the plant chemical space (Poelman & Kessler, 2016). Indirect effects, and particularly those mediated by plants, may even explain species persistence in a community and ultimately the forces that maintain diversity (Veen et al., 2005; Ives & Carpenter, 2007; Estes et al., 2011; Sanders et al., 2013). For example, herbivores may prevent competitive exclusion by creating their own ecological niche through manipulation of plant physiology (Liu et al., 2023). By combining experiments and theory, a recent study tested whether long-term coexistence of the two closely-related herbivorous mites, *T. urticae* and *T. evansi,* was possible because the inferior competitor *T. urticae* was able to manipulate plant physiology for its own benefit (Fragata et al., 2022). Even if this study found that spatial segregation, and not manipulation of plant physiology was the mechanism allowing coexistence, it shows the interest of community ecologists to study herbivore interactions through the lens of plant-mediated indirect effects.

My interest for plant-mediated interaction was fuelled during my postdoc in the Netherlands, where a large part of the laboratory I worked was interested in this topic. During that time, I was part of a European network in which many scientists were also interested in plant-mediated indirect effects. This network was funded under the umbrella of the COST Action FA1405 "Using three-way interactions between plants, microbes and arthropods to enhance crop protection and production" [5]. I was an active member in this community, I was involved in the writing of the proposal and coordinator of the short-term scientific missions. This latter role implied a lot of exchanges with students as I was reviewing their applications to fund short visits between the laboratories of the network. During this period I was involved in the writing of two review articles that dealt with indirect plant-mediated interactions, and specifically on the role of plant phytohormones in mediating plant-insect-microbe interactions. In addition to insect herbivores, we considered microbes, and plant pathogens in particular, because they also trigger defensive plant responses that depend on phytohormones. I wrote the first review in collaboration with David Giron on the role of cytokinins, a group of phytohormones that, relative to jasmonic and salicylic acid, are little studied (Giron et al., 2013). In this comprehensive review we explored how these phytohormones are targeted (and used) by many organisms that colonise plants as parasites, pathogens, herbivores, or mutualists. We also discussed how manipulation or synthesis of cytokinins can reprogram plant physiology with cascading consequences for the community of organisms associated with plants. The second review was in collaboration with PhD student Jenny Lazebnik (Lazebnik et al., 2014). I

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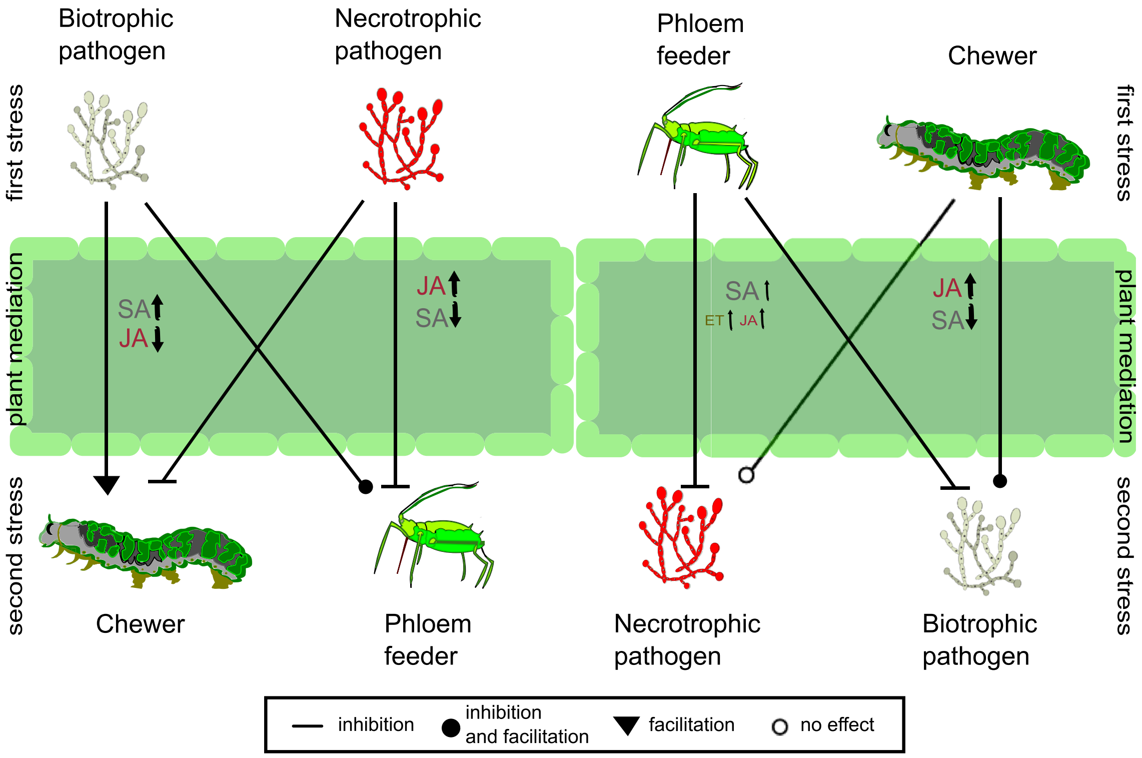

Figure 01. Overview of plant-mediated effects of pathogens on insects and of insects on pathogens of different trophic strategies or feeding modes; including hypothetical phytohormone-mediated mechanisms. Arrow endings represent findings from references discussed in Lazebnik et al. (2014). Acronyms shown as follows: SA = Salicylic acid, JA = Jasmonic acid, ET =Ethylene, ETI = Effector triggered immunity. Modified from Lazebnik et al. (2014).

collaborated with Jenny during my time as postdoc in the Netherlands while she was doing her PhD under the supervision of Joop van Loon. I was not the official PhD supervisor, but for these two reviews I was the person that interacted with her on a daily basis. In this review we proposed a conceptual model of plant-mediated indirect interactions between herbivores and plant pathogens through plant phytohormones, and particularly through the pathways dependent on jasmonic and salicylic acid (Figure 01). The fact that herbivores and pathogens interact through changes in plant physiology or defensive state was known long before we published the study (Stout et al., 2006). Our model, however, moved from reporting patterns to proposing hypotheses on the molecular mechanisms likely to operate in these interactions. This model was based on the idea that early herbivory by a given species (an insect or a pathogen) will trigger a specific phytohormonal response dependent on species guild with consequences on subsequent attackers. By species guilds we considered whether herbivores were sap-suckers or leaf-chewers, or whether pathogens were biotrophic or necrotrophic (i.e. whether they derive nutrients from plant cells, or by killing them). We hypothesised negative impacts between species triggering similar responses so that the second species finds a plant in an already-defended state. We expected, however, facilitation between species belonging to different guilds due to the resource allocation trade-off mentioned above. This model was based on solid evidence from laboratory studies back in 2017, and has been well accepted by the scientific community. Recent evidence, however, suggests that the crosstalk between jasmonic and salicylic acid is a reductionist approach to explain plant responses to multiple herbivores in natural ecosystems. In a recent study with black mustard plants *Brassica nigra* that combined field and laboratory data, Mertens et al. (2021) have shown that plants tailor their responses depending on the community of herbivores that prevails in the field. Plants do not compromise their defensive status after a primary attack, if the second attacker is common in the community the plant is embedded within. Despite this example contradicting some previously accepted general patterns, the crosstalk between jasmonic and salicylic acid has been important to understand herbivore community ecology from a bottom-up approach. As I will explain later, this crosstalk has also been important to understand why some insects associate with bacterial symbionts to manipulate plant physiology for their own benefit.

b. Plants respond to insect oviposition

Plants are sessile organisms and at the vegetative state they can not disperse to avoid herbivores or pathogens. As I have explained along this section, plants have evolved very elaborate induced defences to counter these attackers. Despite these defences, most individual plants suffer substantial herbivory mainly because herbivores can withstand toxic metabolites or physical defences, resist natural enemies that plant volatiles attract, or evolve counter-defensive measures (Schoonhoven et al., 2005). Even if induced defences were extremely efficient, they are usually induced when the herbivore is already feeding. Defending the plant when herbivores are already munching on leaves is a risky strategy, and this is why some plants have evolved ways of detecting eggs as early cues of herbivory. Insect oviposition has been found to induce plant responses in at least 20 insect species including beetles, moths, butterflies, plant- and leafhoppers, sawflies, and flies (Hilker & Fatouros, 2015). These defences target the egg directly, for example through the formation of plant tissues that crush eggs, or the production of ovicidal substances. Eggs can also induce changes in plant secondary metabolites that target the emerging neonate, or trigger the emission of plant volatiles that attract enemies of the developing young (Cusumano et al., 2015; Hilker & Fatouros, 2015, 2016). Egg-induced responses are therefore common in plants, but are they relevant for the structure of herbivore communities? In a study in collaboration with PhD student Foteini Pashalidou we performed a field experiment to explore this question (Pashalidou et al., 2015). I was involved in this project during my postdoc in the Netherlands while Foteini was doing her PhD under the supervision of Nina Fatouros. In this study another PhD student Eddie Griese (also supervised by Nina) also assisted in the field work. We worked with the large white butterfly *P. brassicae*, which is known to trigger in some plants an egg-killing response. To kill *P. brassicae* eggs, plants trigger a so-called hypersensitive response whereby plant cells surrounding the egg necrose and eggs dessicate eventually dropping from the plant (Blaakmeer et al., 1994; Balbyshev & Lorenzen, 1997). In our study we exposed large white butterfly *P. brassicae* larvae

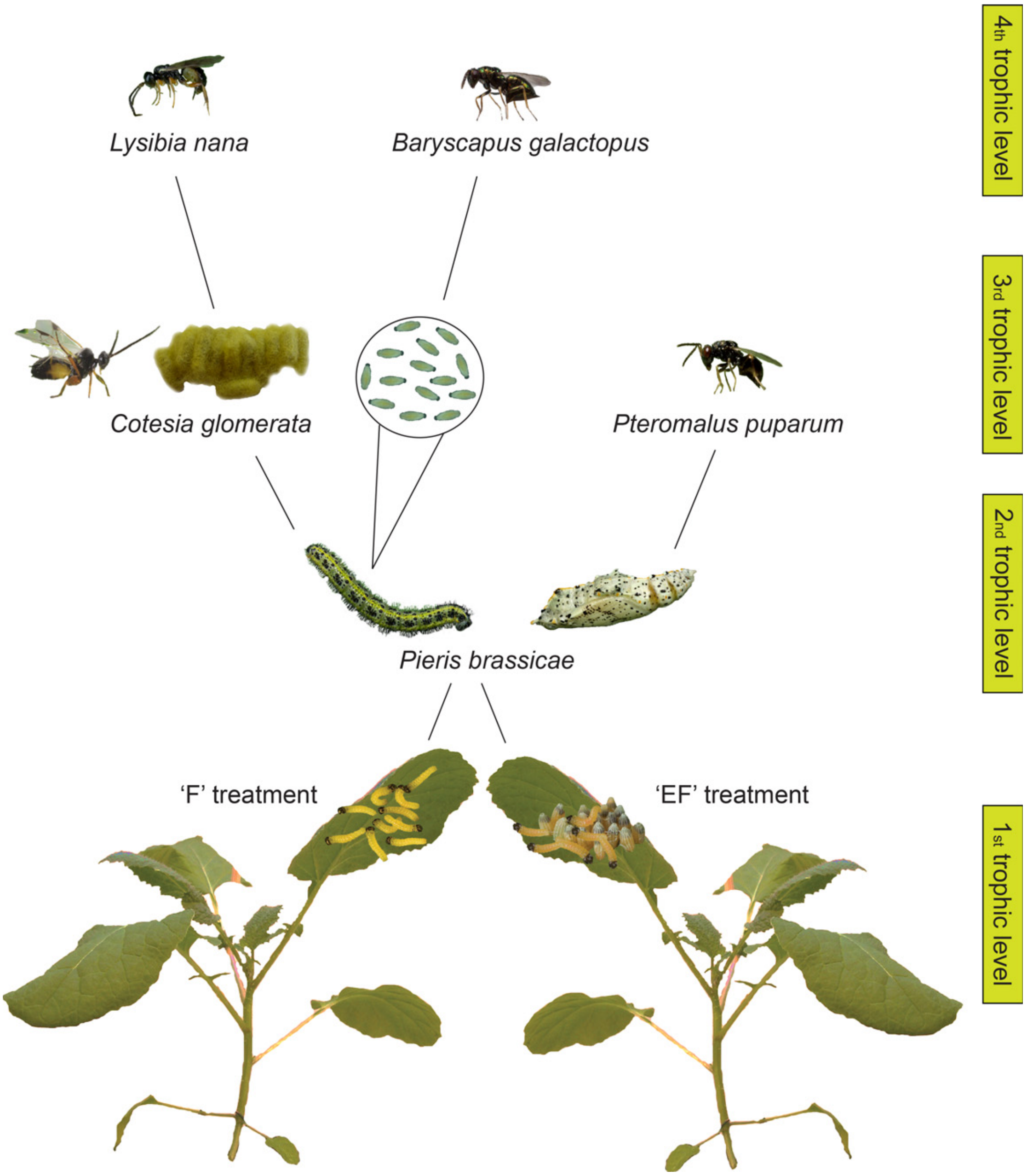

Figure 02. Trophic web on *Brassica nigra* plants studied in the field experiment in Pashalidou et al. (2015). Primary parasitoids of the third trophic level attack the caterpillars (i.e. the gregarious endoparasitoid *Cotesia glomerata*) and pupae (i.e. the gregarious endoparasitoid *Pteromalus puparum*) of the large cabbage white butterfly *Pieris brassicae* of the second trophic level. The larvae of the primary parasitoid *C. glomerata* inside the herbivore host are attacked by the hyperparasitoid *Baryscapus galactopus* and *C. glomerata* cocoons are attacked by *Lysibia nana*, both wasps belonging to the fourth trophic level. The effects of the two different treatments were tested on the performance and the parasitisation rate of insects at the second, third and fourth trophic levels. **EF** plants were exposed to *P. brassicae* **E**gg deposition and subsequent larval **F**eeding (plant on the right) and **F** plants were exposed to larval **F**eeding only (plant on the left). Photo credits: www.bugsinthepicture.com. Modified from Pashalidou et al. (2015).

feeding on black mustard *Brassica nigra* plants to the natural community of natural enemies. We induced defences of half the plants by allowing adult butterflies to lay eggs on them (Figure 02). Once in the field, we recovered caterpillars and pupae as they became parasitised, and all along the experiment we weighted caterpillars, butterfly pupae and adult parasitoids. We also estimated parasitism rates on larvae and pupae on the two different types of plants. This experiment revealed a lot of interesting ecological interactions. (i) In agreement with our initial hypotheses, egg induction reduced the weight of *P. brassicae* caterpillars and pupae. Insect size usually correlates positively with fitness. Particularly for females, larger individuals can survive for longer and lay more eggs. Egg-induction of plant defences thus had a direct protective effect for plants by reducing the fitness of their attackers, and likely the amount of plant consumed. (ii) Egg induction had a cascading effect that reached up to higher trophic levels: both *P. brassicae* parasitoids and hyperparasitoids were also of smaller size. If indirect effects are usually reported as interactions between two species mediated by a third one, in this case egg oviposition had an indirect effect on hyperparasitoids through three species: the plant, the caterpillar and the primary parasitoid. (iii) Egg induction increased parasitism rates on caterpillars so that egg-induced plant volatiles likely played an indirect defensive role. A recent report by some of the same authors of this previous study has recently shown even more subtle interactions. In response to egg oviposition by the large white butterfly *P. brassicae,* black mustard plants *B. nigra* triggered the emission of volatiles that informed neighbouring plants of risk of herbivory (Pashalidou et al., 2020). Such information rendered receiving plants more resistant to herbivores, and triggered a developmental shift in plants from growth to reproduction. I started this paragraph by saying that plants are sessile and can not easily escape their enemies, but this later study is one of many (e.g. Bont et al., 2020) in which plants do so by increasing seed production in response to herbivory.

The studies presented in this section reveal the importance of considering the wider community context to understand how plant defences impact herbivore population dynamics. Herbivore-induced plant volatiles, for example, were first described in laboratory experiments in which parasitoid preference was tested for plants that were attacked or not by herbivores (Dicke & Sabelis, 1987; Turlings et al., 1990). Attraction of enemies by infested plants was thought to be the paramount of defences. Once in the field, however, the defensive value of these volatiles became less clear because they did not always increase plant Darwinian fitness, i.e. increased successful reproduction. Plant defences can backfire if volatiles are used by herbivores to detect plants, or if the volatiles attract hyperparasitoids or enemies that are not able to attack the herbivore triggering the defence (Karban & Baldwin, 2007; Dicke & Baldwin, 2010; Kessler & Heil, 2011; Schuman et al., 2012). I realised the multifaceted nature of plant volatiles in a field study that I will explain in detail in the following section (Frago et al., 2022). We found that instead of attracting natural enemies, plant volatiles altered the physiological state of neighbouring plants with an unclear benefit for the emitting plant. These unexpected effects make the use of induced plant defences for applied interests complicated, and call for joint efforts between plant ecologists and pest managers. Thanks to methodological advances in the analysis and synthesis of plant metabolites, synthetic molecules are increasingly used to induce defences in crops (Turlings & Erb, 2018; Brilli et al., 2019; Del Buono, 2021). This was in fact the ultimate goal of a project I have been involved in from 2019 to 2022: PRIMA INTOMED "Innovative tools to combat crop pests in the Mediterranean" [6] . In this collaborative consortium, we identified candidate metabolites derived from induced plant defences. These metabolites were then tested as toxic chemicals against pests, as inducers of plant defences, or as attractants of natural enemies, with the final aim to test them in field trials. Plant-based biopesticides are already common in the market, but products inspired on what plants produce when triggering defences are still rare (Turlings & Erb, 2018; Brilli et al., 2019; Divekar et al., 2022). In agriculture, the use of synthetic volatiles that attract pest enemies, for example, has been discussed almost since the beginning of their discovery, but serious attempts to use them are more recent (Pickett & Khan, 2016). There are more failures than success stories. Synthetic volatiles are highly reactive and degrade quickly, and their mass production and marketing is complex making their prices unaffordable for most farmers (Brilli et al., 2019). Once these technical caveats are solved, triggering crop defences using molecular biopesticides may become widespread. Community ecology approaches will again play an important role in these advances to ensure that novel products are innocuous to beneficial and non-pest arthropods. Considering the wider community of pests can also prevent that the chemicals used trigger defences against a specific pest, but at the same time benefit other pests through intricate trade-offs among the different plant defence lines.

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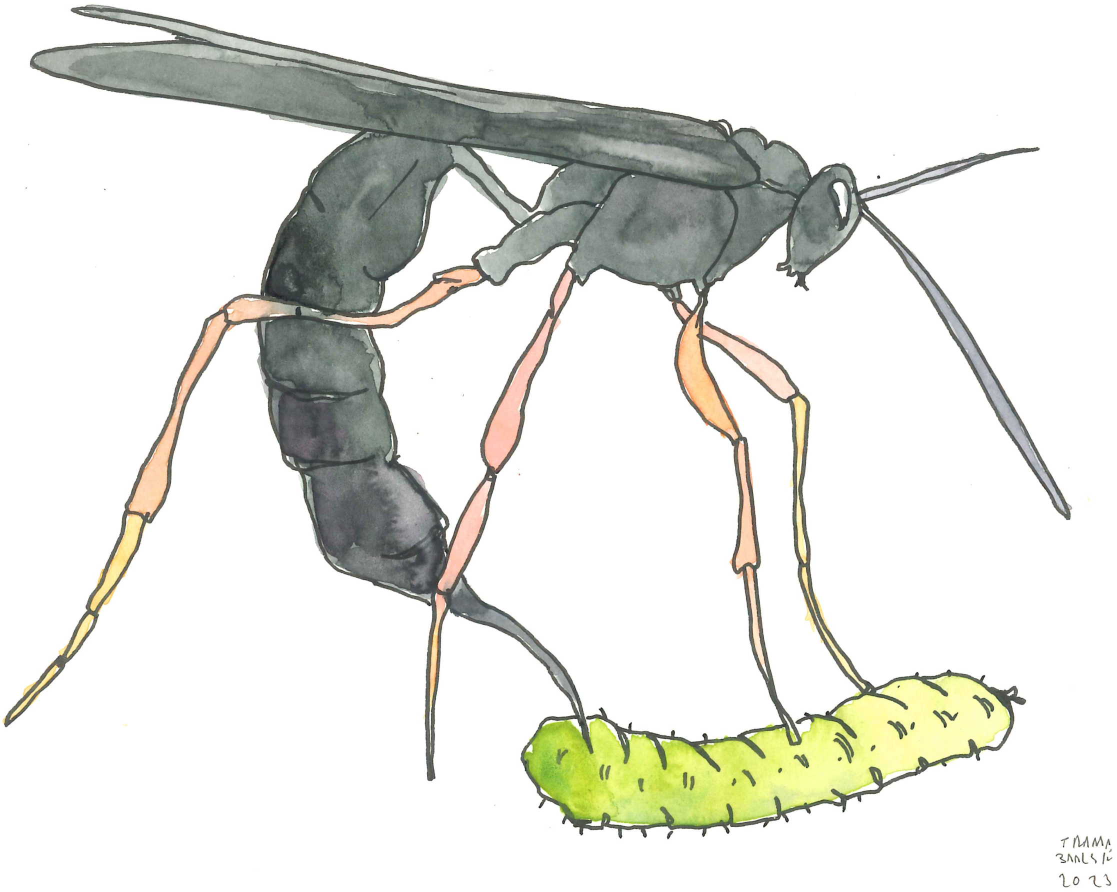
CHAPTER 2: COMPLEX INTERACTIONS AMONG INSECT NATURAL ENEMIES



The parasitoid *Cotesia glomerata* laying an egg in a *Pieris brassicae* caterpillar (watercolour on paper by tramabarcelo)

2.1. CONTEXT

The fact that plants have evolved a system as complex as the emission of herbivore-induced plant volatiles to enhance top-down control over herbivores (as discussed above) is good evidence of the importance of natural enemies as plant allies. Unintentional introductions of insects in exotic habitats are good natural experiments that revealed the importance of such allies (Caltagirone, 1981). When invasive species colonise new geographical areas they often find ecosystems devoid of their natural enemies, which allows them to outbreak with dramatic consequences for plants. Examples from classic biological control programs in which invasive species have been controlled by translocating their enemies from the native area to the exotic one also provide good evidence of the suppression potential of natural enemies. A classic example comes from the Cottony cushion scale, *Icerya purchasi*, a tropical species that invaded California around 1868. Less than 20 years later, the species exploded causing devastating effects on the citrus industry. The pest was miraculously suppressed to low levels a few years after thanks to the introduction of the ladybird *Novius cardinalis*, which replaced orange trees covered in leprous-like scales by swarms of ladybirds (DeBach, 1974). Herbivore-enemy interactions, however, are rarely that simple and magic bullets to control pests are rare. Many biocontrol programs fail because enemy networks are extremely intertwined (Caltagirone, 1981; van Lenteren et al., 2006). Many natural enemies may be needed to control a pest, enemies can kill each other instead of attacking pests via intraguild predation, or they can switch to alternative hosts that are not pests. Taking a community ecology approach is therefore important to practise sound biocontrol (e.g. Denoth et al., 2002a; Symondson et al., 2002). An important part of my research has been devoted to providing fundamental ecological knowledge useful to understand the dynamics of herbivore communities, but also to control pests. As I will show below, developing general principles to implement successful biocontrol would not be possible without ecological theory, but such theory needs to be nourished from biocontrol practice.

Good examples of the porous boundary between applied and fundamental ecology come from two of the ecological interactions I have been interested in: intraguild predation and apparent competition. As briefly defined before, intraguild predation occurs when two enemies that prey on each other also compete for (or share) a prey resource. Apparent competition is an indirect interaction that occurs when two herbivores interact through a shared natural enemy (Figure 03). In arthropod communities this interaction is common because one herbivore can increase predation or parasitism on another, because its presence results in an increased abundance of a shared natural enemy (van Veen et al., 2006; Morin, 2011). Intraguild predation and apparent competition are ubiquitous in trophic webs, and as I will explain later, they are key to understanding the dynamics of insect communities. Ecologists have set-up solid theoretical grounds for these two interactions thus making an easy appreciation of these interactions in biocontrol programs (Polis & Holt, 1992; Rosenheim, 1998; van Veen et al., 2006). Intraguild predation, for instance, was long observed (and feared) by biocontrol practitioners: if enemies eat each

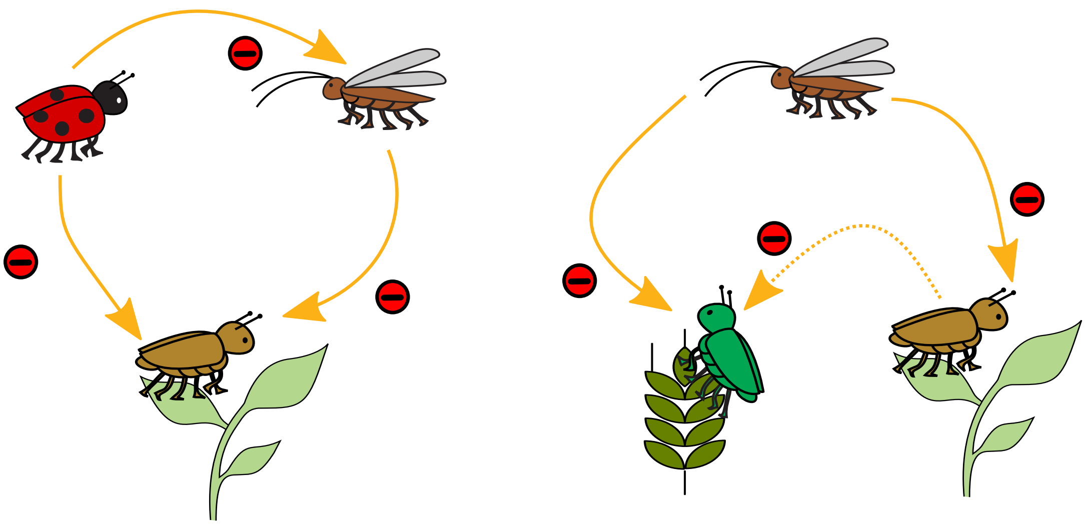


Figure 03. Schematic representation of intraguild predation (left) and apparent competition (right) between herbivores (brown or green), a ladybird and a parasitoid. Solid arrows point from predator to prey. Intraguild predation is asymmetrical in this example so that the ladybird can feed on the parasitoid but not vice-versa. The dashed line represents apparent competition, an indirect effect between herbivores mediated by the parasitoid.

other predation on pests may be dampened. A clear delimitation of the term, however, was not proposed until 1995 (Rosenheim, 1995). Apparent competition, on the other hand, was described theoretically back in 1977 (Holt, 1977) but the interest in designing landscapes that promote apparent competition between pests and alternative hosts of pest natural enemies is more recent (Deguine et al., 2017). These alternative hosts may increase enemy density, or allow enemy persistence in crop margins so they can spillover to crops when pests arrive. Despite many theoretical advances and a growing body of information available, biocontrol practitioners do not always fully appreciate the importance of complex indirect interactions like intraguild predation or apparent competition. This appreciation is important to better understand pest dynamics because whether insect live in pristine forest or agricultural lands, natural enemy impacts can rarely be predicted from pairwise species interactions (Sih et al., 1998; Veen et al., 2005; Ives & Carpenter, 2007; Estes et al., 2011).

2.2. HYPERPARASITISM AND PARASITOID COMPETITION

During my PhD I studied the complex of natural enemies associated with the browntail moth *Euproctis chrysorrhoea*, a forest pest. Relative to other forest pests in Europe, *E. chrysorrhoea* had been little studied and I quickly realised that the community of natural enemies associated with this species was much more diverse and complex than we expected. We studied the natural history of this moth in four populations in eastern Iberian Peninsula, and our list of natural enemies became as long as 26 parasitoid species and few predators (Frago et al., 2011, 2012b). We particularly focused on parasitoids, and found that the parasitoid complex of *E. chrysorrhoea* was not only diverse in species, but also very rich in interactions. As Matryoshka dolls, primary parasitoids of the moth were attacked by secondary parasitoids (or hyperparasitoids) (Frago et al., 2012b), and we even discovered that the parasitoid *Pediobius pyrgo* was capable of acting both as secondary and as tertiary parasitoid (i.e. it was found attacking a secondary parasitoid) (Frago et al., 2010) (Figure 04).

Parasitoids have greatly contributed to the development of ecological and evolutionary theory. They are, for example, an excellent group for the study of interspecific competition. They are very diverse and different stages of the same host species (e.g., eggs, larvae, pupae) often harbour different parasitoid communities. Unlike arthropod predators, which may require many prey to achieve maturity, parasitoid development is constrained to a single host that is often not much larger than the adult parasitoid. This implies that parasitoids fight fiercely for host resources, particularly those species in which a single host allows the development of a single adult parasitoid (Harvey, 2005; Harvey et al., 2013). Such tight competition for hosts is suggested to be at the core of parasitoid diversity (May & Hassell, 1981; Murdoch & Briggs, 1996; Bonsall & Hassell, 1997; Borer, 2002; Borer et al., 2003) because according to the competitive exclusion principle, to coexist on similar resources parasitoids need to diverge at least on one trait (Hardin, 1960; DeBach, 1966; Chesson, 2000). The predicted parasitoid competition and its effects on parasitoid diversification has been validated in laboratory experiments, but direct demonstration of parasitoid competition in the field is elusive. The mechanisms favouring coexistence include specialization in resource use, temporal and spatial resource partitioning, or subtle variations in environmental conditions (Chesson, 2000; Amarasekare, 2003; Snyder et al., 2005; Schoener, 2011). In collaboration with Tolis Pekas I led a study that proposed partitioning of host sizes as a novel mechanism by which parasitoids may be able to coexist (Pekas et al., 2016). We studied two parasitoids of an important citrus pest, the California red scale *Aonidiella aurantii.* These parasitoids belong to the genus *Aphitis*: *Aphitis melinus* and *A. chrysomphali.* These two species exploit the same host stages and both prefer larger hosts, but despite such strong competition they coexist in sympatry (i.e. in the same area) even if *A. melinus* is a stronger competitor. By sampling insects along 12 replicated orange groves we demonstrated that one way *A. chrysomphali* is able to persist

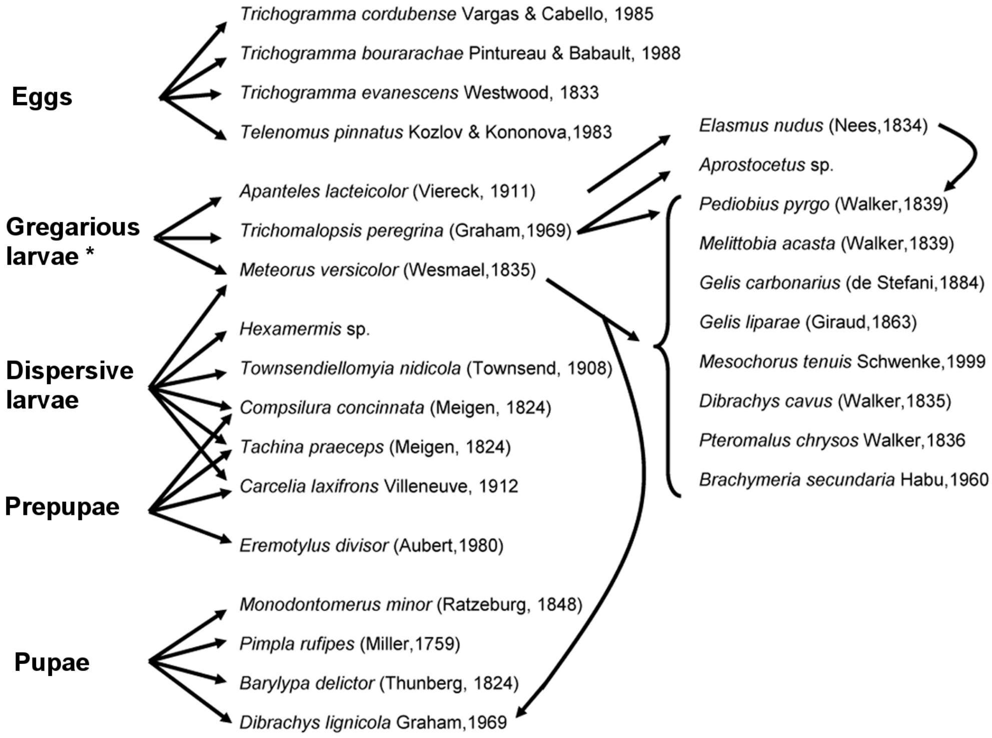


Figure 04. The parasitoid complex of *Euproctis chrysorrhoea* in the eastern Iberian Peninsula. Arrows indicate trophic links pointing from host to parasitoid. Modified from Frago et al (2012b).

is by exploiting hosts in a plastic way: the poorer competitor switches to low-quality small hosts at larger densities of the stronger competitor *A. melinus.* This study added novel evidence on the importance of species traits, and in particular body size, in mediating species interactions [e.g. Schneider et al. (2012)]. It also revealed that even if host size often correlates with reproductive success in parasitoids (Godfray, 1994; Harvey, 2005), selection can favour utilising smaller hosts if this increases the chances of reaching adulthood. Another example of the importance of bringing the lens of community ecology to understand species interactions.

2.3. ENEMIES SHARING PREY: APPARENT COMPETITION

Even if herbivores compete for plant resources, apparent competition is thought to be one of the prime forces determining the structure of herbivore food webs (van Veen et al., 2006). Apparent competition was first proposed theoretically (Holt, 1977), proved in the laboratory with experiments with simple communities (Bonsall & Hassell, 1997) and then demonstrated in the field (Morris et al., 2004; Frost et al., 2016). An interesting example of the power of apparent competition in the dynamics of insect herbivores comes from the spongy moth *Lymantria dispar*, which travelled from Europe to invade and outbreak in America in 1869. Efforts to control the species through classical biocontrol failed and even backfired particularly due to the introduction of the generalist fly *Compsilura concinnata* in 1906 (Howard & Fiske, 1911). This fly tracked *L. dispar* during its invasion across the continent, but it did not control its populations. This generalist parasitoid, however, became very abundant in areas with large densities of the invasive moth, often spilling over from its primary host and causing declines through apparent competition of local emblematic butterflies like giant silk moths (Redman & Scriber, 2000; Elkinton & Boettner, 2004).

As the example above shows, apparent competition commonly occurs when one herbivore impacts another through increased densities of a shared natural enemy. This effect is density-mediated, but trait-mediated apparent competition is also common when the interaction is transmitted through changes in species behaviour (Abrams, 1995; Abrams et al., 1996; Werner & Peacor, 2003; Schmitz et al., 2004). Common examples of trait-mediated effects triggered by natural enemies occur when the mere presence of a natural enemy triggers avoidance behaviours in herbivorous prey, which reduce feeding. In this example, the enemy has a positive trait-mediated effect on the plant through reduced herbivore feeding (Schmitz et al., 2004; Thaler et al., 2012b). I have already discussed that one way plants defend from herbivores is through the emission of herbivore-induced plant volatiles that attract herbivore's natural enemies. This is a trait-mediated indirect effect whereby herbivores change natural enemy behaviour through changes in the plant. We have a deep understanding of the molecular routes by which herbivore-induced plant volatiles are produced (e.g. Webster & Cardé, 2017; Turlings & Erb, 2018; Ali et al., 2023). This contrasts with the little knowledge we have on volatile effects on parasitoid behaviour (or trait-mediated effects) in the field and their consequences at a larger community context (but see De Moraes et al., 1998; Xiao et al., 2012). To fill this gap, we recently published a study where we explored how herbivore-induced plant volatiles alter apparent competition between herbivorous insects (Frago et al., 2022). We explored how the population dynamics and fitness of the English grain aphid *Sitobion avenae* feeding on wheat *Triticum aestivum* was affected by volatiles emitted by broad beans *Vicia faba* plants upon infestation by the pea aphid *Acyrthosiphon pisum*. Our hypothesised trait-mediated apparent competition effect was that the attacked plant would emit volatiles that would attract aphid natural enemies. These volatiles would increase the local density of aphid natural enemies leading to an increase in parasitism on the other aphid species located nearby. This study took a long time to get published because each of the successive experiments did not validate our expectations, but opened the door to a new one. In experimental ecology, experiments that provide a clear answer are more the exception than the norm, but this study was an extreme case of the pattern. We started with a field experiment where we followed the dynamics of *S. avenae* aphids feeding on wheat. Wheat plants were surrounded by beans that had previously experienced feeding by *A. pisum* aphids or not. We knew from previous studies that infested *V. faba* plants emit herbivore-

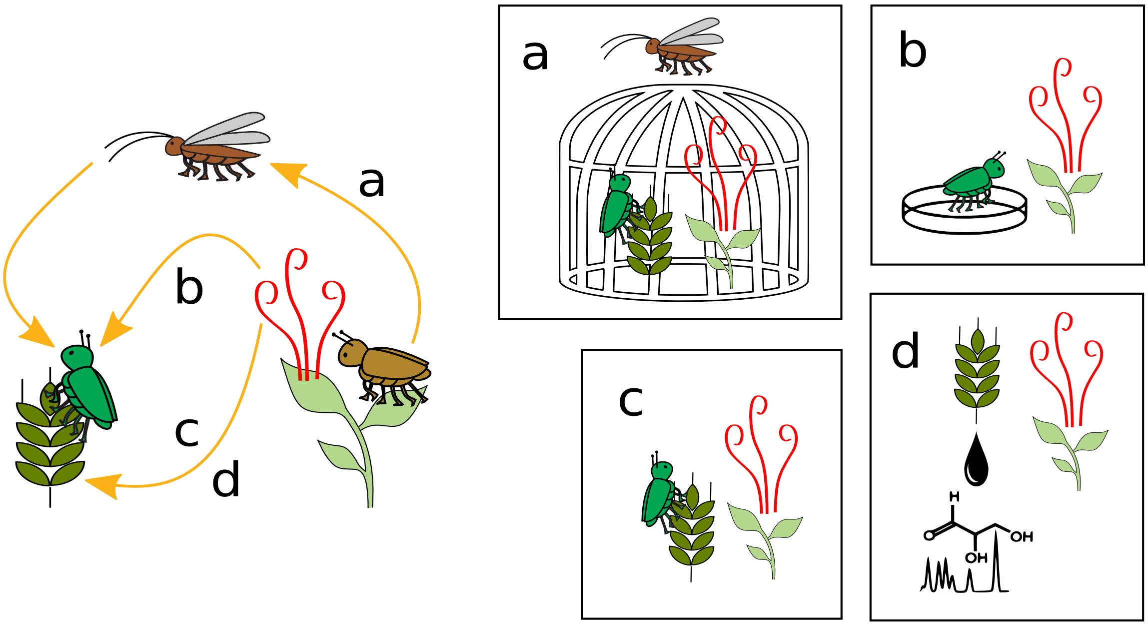


Figure 05. Schematic representation of the different interactions tested and the experiments performed in Frago et al (2022). Herbivore-induced plant volatiles (represented by red lines) emitted by bean *Vicia faba* plants fed upon by pea aphids *Acyrthosiphon pisum*, represented by a brown insect, may modulate the population dynamics of grain aphids *Sitobion avenae*, represented by a green insect, feeding on wheat *Triticum aestivum* (a) indirectly through shared natural enemies (apparent competition), (b) through direct volatile effects on aphids, and (c) indirectly through volatile effects on wheat physiology (d). These interactions have been tested (A) in the field by assessing the population dynamics of *S. avenae* aphids and its natural enemies, in an experiment that crossed volatile emissions and natural enemy exclusion treatments. Glasshouse experiments tested volatile effects on *S. avenae* aphids feeding on either (B) an artificial diet, or (C) on wheat plants. (D) Volatile effects on wheat physiology were explored by analysing wheat phloem exudate composition. Dashed lines represent indirect interactions. Modified from Frago et al (2022).

induced volatiles that attract aphid natural enemies (Du et al., 1996, 1998; Guerrieri et al., 1999; Takemoto & Takabayashi, 2015). The volatile treatment was crossed with a treatment that excluded natural enemies in the field. This two by two design was needed to test whether volatile emissions were truly altering *S. avenae* dynamics through changes in natural enemy attraction. It turned out that the tedious effort of setting up this control with heavy wired cages was justified. Bean plant volatiles did not attract natural enemies, but had a positive effect on *S. avenae* aphids in plots where natural enemies were excluded (Figure 05). Subsequent laboratory experiments and metabolomic analyses revealed that *V. faba* volatiles had indeed a positive effect on *S. avenae* aphids, probably through changes in wheat physiology (Figure 06). We therefore reported a mutualistic interaction between aphids through plant-plant communication, instead of the expected natural enemy-mediated antagonism.

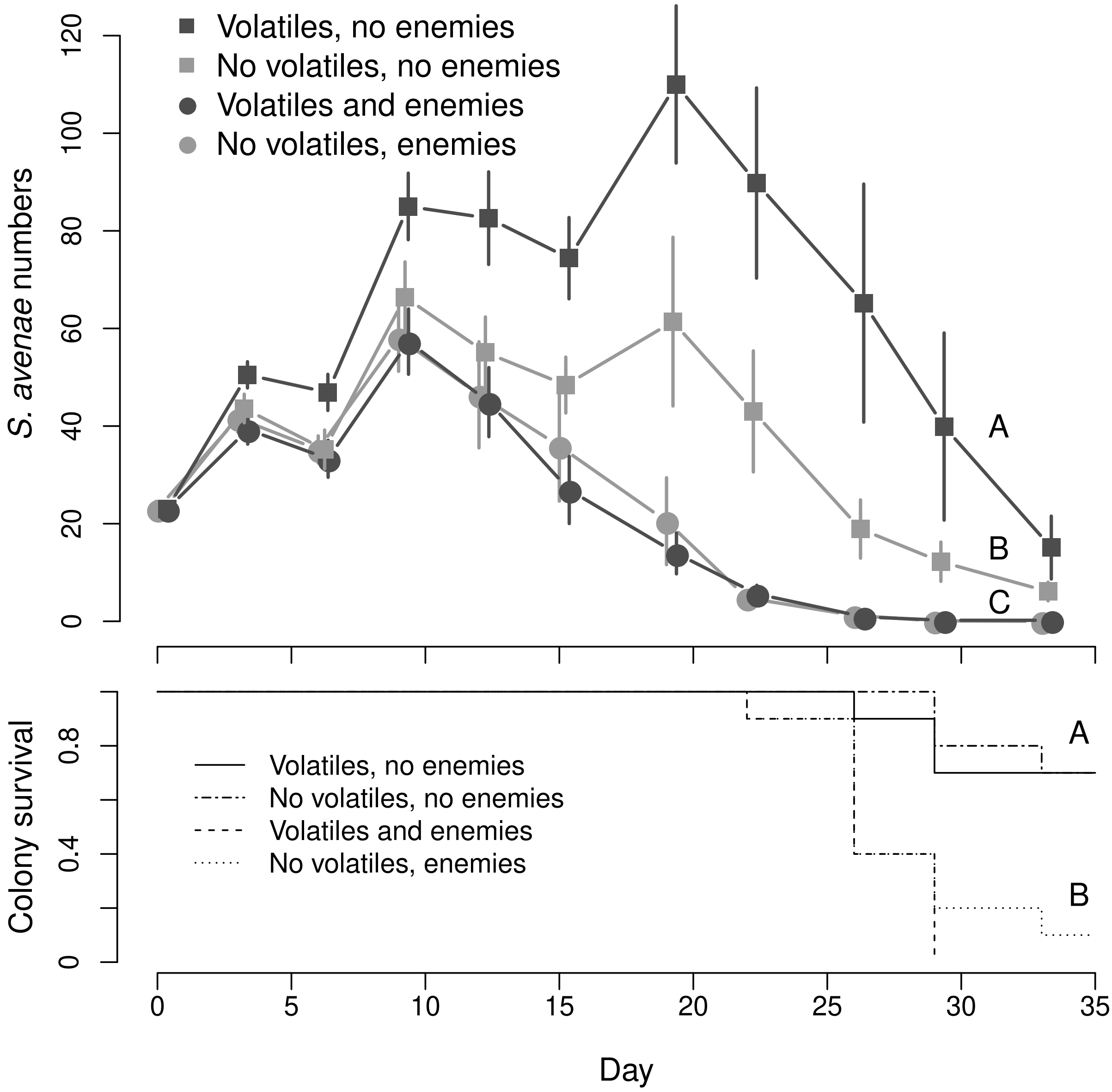


Figure 06. Population dynamics (top) and colony survival (bottom) of *Sitobion avenae* aphids in the field experiment in Frago et al (2022). The mean size of aphid colonies (± SE) and proportion of aphid colonies that survived (n=10) over the course of the experiment are presented. Colonies of *S. avenae* aphids feeding on *Triticum aestivum* were surrounded by *Vicia faba* plants previously infested with *Acyrthosiphon pisum* aphids or not (volatile treatment). This treatment was crossed with natural enemy exclusion (enemy treatment) in a factorial design. For each panel, different letters at the end of the curves represent significant differences in aphid numbers or colony survival among plot types ($p < 0.05$ based on Tukey posthoc test and pairwise comparisons between treatments on aphid numbers and colony survival, respectively). In the population dynamics plot, posthoc tests are based on mixed effects models for aphid numbers through time. Modified from Frago et al (2022).

Most examples of plants responding to herbivore-induced plant volatiles emitted by neighbouring plants report increased resistance, i.e. volatile-mediated associational resistance. This effect would tend to depress herbivore numbers when nearby plants are already attacked so that plants use volatiles as early warning cues to trigger defences (Erb et al., 2015; Moreira & Abdala-Roberts, 2019; Timilsena et al., 2020). As with volatile attraction of herbivore natural enemies, associational resistance can be very specific, as found in the wild plant *Baccharis salicifolia* (Moreira et al., 2018). Feeding by the aphids *Uroleucon macolai* and *Aphis gossypii* triggered volatiles that made other plants more resistant but only to the same aphid species responsible for volatile induction. Our study, however, reported the opposite. In response to infected plants in the vicinity, plants suffered increased herbivory (i.e. larger aphid densities), an example of associational susceptibility. Our study showed that interaction networks can be very complex, particularly when plant volatiles are considered. Even if the last decades have shown remarkable progress in the study of trophic webs, more experimental work is needed to fully appreciate the importance of indirect interactions in plant, herbivore and natural enemy trophic webs. Our understanding on how these networks are altered by trait-mediated effects, or by plant volatiles in particular, can also be very useful to elucidate which plant combinations lead to associational resistance or susceptibility in croplands. This information will be useful to design ecological crop protection strategies.

2.4. ENEMIES EATING EACH OTHER: INTRAGUILD PREDATION

In previous sections I have shown that parasitoids are killed by their own kin. Miserable as this might be, they are also eaten by intraguild predators. Intraguild predation has been central in my research during the last few years, and it will continue being so during the years to come. Intraguild predation can be symmetrical when both enemies can eat each other, but asymmetrical interactions whereby only one predator feeds on the other are more common (van der Hammen et al., 2010; Montserrat et al., 2012; Fonseca et al., 2018; Marques et al., 2018). Parasitoids suffer from such asymmetry because in their interactions with predators they are always the subordinate species. Parasitized and non parasitized hosts are often equally vulnerable to predation so that when predators eat a parasitized prey they unnoticeably eat the parasitoid larva developing inside (Brodeur & Rosenheim, 2000; Frago, 2016). I just discussed above an example of how parasitoids can avoid competitive exclusion by exploiting small hosts that are less preferred by stronger competitors. Parasitoids have also evolved strategies to avoid intraguild predators, for example by avoiding laying eggs in host patches where they detect intraguild predators. An overview of these strategies can be found in a review that I wrote as a single author (Frago, 2016). Among these strategies, a well studied one is that of the parasitoid *Aphidius ervi*, which avoids patches containing chemical signals derived from the ladybird predator *Coccinella septempunctata.* This behaviour has important consequences for parasitoid population dynamics because in the presence of these traces the parasitoid reduces attack rates on aphids (Nakashima et al., 2004, 2006). In a study that I did during my first postdoc with Charles Godfray, we explored the community-wide consequences of this effect (Frago & Godfray, 2013). To do so, we worked with a community composed of the aphids *A. pisum* and *S. avenae*, the parasitoid *Aphidius ervi* and the seven-spot ladybird *Coccinella septempunctata.* We replicated this community in population cages, and we studied its dynamics over several aphid generations. The two aphids *A. pisum* and *S. avenae* fed on, respectively, broad beans *V. faba* and wheat *T. aestivum* so they did not compete directly for the plant resource. In our study, however, their dynamics were coupled through apparent competition through the shared parasitoid. Following the methods proposed by (Nakashima et al., 2004, 2006), we extracted *C. septempunctata* ladybird chemicals that we subsequently sprayed on bean plants just before starting the experiment. As expected, parasitoids avoided these plants, and as such *A. pisum* aphids experienced lower parasitism rates. This initial reduction in parasitism on one aphid species had lasting consequences for both aphid species. In cages where we sprayed ladybird chemical cues, parasitoid numbers were lower and both aphid species became more abundant after several generations. *S. avenae* thus benefited from the presence of a dominant intraguild predator foraging on another species of aphid (*A. pisum*) on a different food plant. This was an example of trait-mediated apparent mutualism (Abrams et al., 1998). As explained above, the effect was trait-mediated because it was transmitted through a behavioural change, in this case the parasitoid avoidance of chemical cues. It was an example of apparent mutualism because the shared natural enemy led to an indirect benefit, as opposed to a negative impact that would have been expected under apparent competition. This study revealed an ecological interaction in which a natural enemy has evolved a sophisticated way to avoid antagonists with far-reaching consequences for the dynamics of an insect community.

This experiment was important for me because it was the first of many where I studied the long-term dynamics (i.e. over several generations) of arthropod communities in population cages. Population cages are commonly used to explore ecological interactions at a microcosm scale. Even if these cages limit dispersal and habitat structure they have been key to validate ecological theory. For instance, apparent competition was first described theoretically, but the first experimental demonstration came from a population cage experience (Bonsall & Hassell, 1997). The fact that in population cages we can monitor the long-term multi-generational dynamics of arthropod communities is also important. First, because with long-term dynamics we can validate the principles arising from theoretical models, whose predictions are based on equilibrium conditions that occur after a multitude of *in silico* generations (Polis & Holt, 1992; Turchin & Taylor, 1992; Bonsall & Hassell, 1997). Second, because long-term dynamics allow us to monitor species extinctions and persistence, which is a key element to understand the forces that maintain diversity [e.g. (Frago, 2016; Sanders et al., 2016; Kehoe et al., 2020)] From an applied point of view, this approach can help us assess lasting biocontrol services, or the persistence of pest natural enemies through time.

Intraguild predation, population cages and long-term dynamics were common themes in the two PhD projects that I co-supervised with Samuel Nibouche during my time as Cirad researcher in Réunion island (a French overseas Department located near Madagascar). These PhD projects were those of Niry Dianzinga and Karim Tighiouart. In Niry's PhD Lala Raveloson Ravaomanarivo from the University of Tana in Madagascar was also Director. Although these two PhD projects were not the first ones I was involved in, they were the first that were based on my own projects. The team "thrips" was quite large at some point consisting of two PhDs, two MSc, one laboratory assistant and myself. The two PhD projects also implied collaboration with the biocontrol company *La Coccinelle* [7]. This company produced natural enemies to be used as biocontrol agents in Reunion greenhouses. At that time, the company was small but one of the first of its kind in the Indian Ocean. Our role was to provide ecological knowledge for a better use of the natural enemies they produced. These projects exposed the young scientists (and myself) to the advantages and constraints of working with the private sector. We had all natural enemies available with no need to rear them by ourselves, we also had expert collaborators on the biology of the species we worked with, but we made some compromises. For example, as herbivores we needed to work with pest species that were not always the easiest to maintain in the laboratory.

Niry Dianzinga's PhD explored the diversity of thrips in Reunion and the efficiency of their natural enemies to control them. In one of his projects Niry and two MSc students (Sandro Moretti and Laura Madeleine) explored the dynamics of a community composed of two species of thrips (*Thrips parvispinus* and *Frankliniella occidentalis*), and two predatory mites (*Amblyseius swirskii* and *Proprioseiopsis mexicanus*). The two thrips species were common in sweet-pepper *Capsicum annuum* greenhouses, and were causing important damages thus risking sweet-pepper production on the island. The two predatory mite species were also abundant in these same greenhouses, but their natural densities were not enough to suppress pest densities. *La Coccinelle* initiated a mass rearing of the two mite species. *A. swirskii* is a well known species, and probably the most widespread mite species used as a biocontrol agent, whereas *P. mexicanus* was a species that had rarely been used for biocontrol purposes (e.g. Momen & El-Saway, 1993; Gerson & Weintraub, 2012). We studied interactions between these four species at different levels: we followed their dynamics in the field, we studied their behaviour in small arenas, and we followed their long-term dynamics in population cages. Many students were involved in this study. Niry Dianzinga supervised most experiments and performed the long-term community dynamics, MSc student Sandro Moretti performed the first predation experiments that provided very useful data on the intraguild potential of the two mite species studied. Laura Madeleine did her MSc project when Niry was in his third year of PhD and she performed behavioural experiments to better understand the results that we obtained the years before. Overall we reported that *A. swirskii* was a superior competitor.

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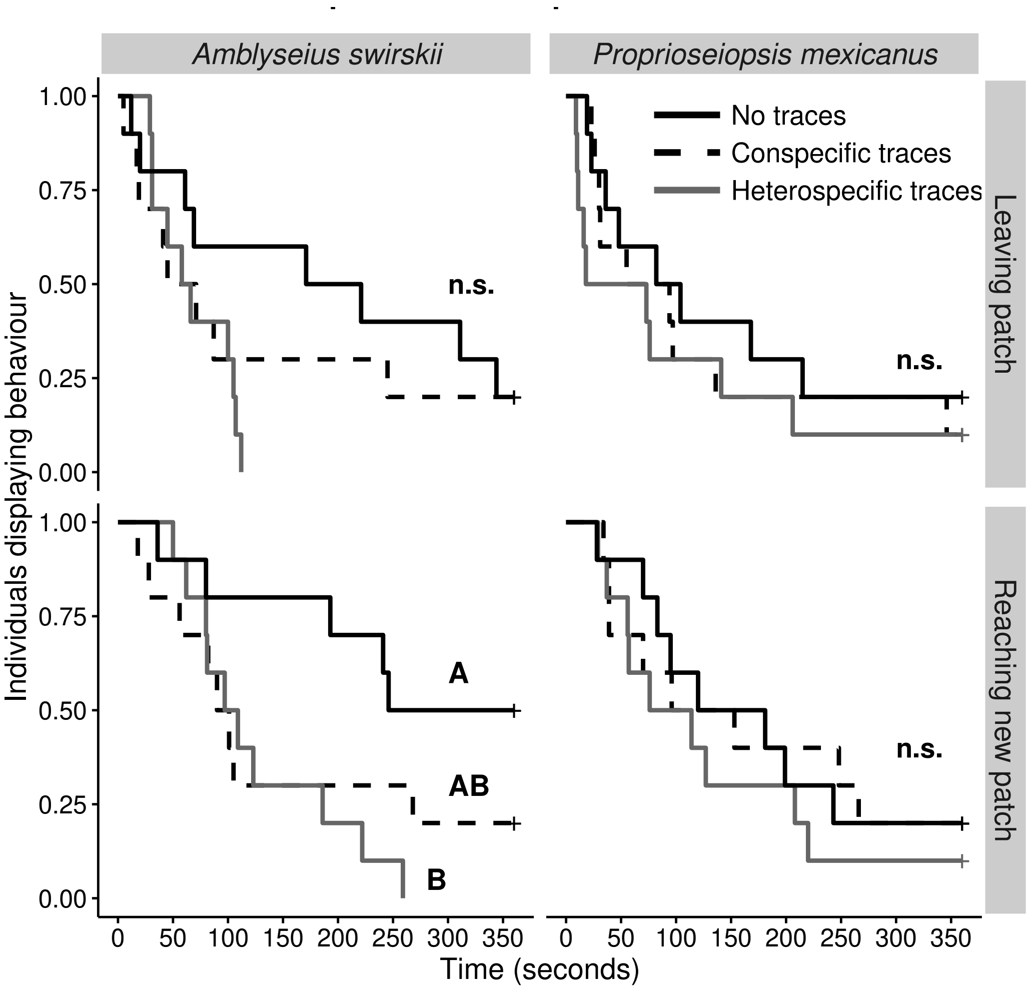

Figure 07. Behavioural experiment exploring the time that predatory mites *Amblyseius swirskii* and *Proprioseiopsis mexicanus* require to disperse by leaving a patch and to reach a new one in the presence of conspecific traces, heterospecific traces or in controls. The y-axis are Kaplan-Meyer time to event curves that represent the time required for the different individuals tested to display the behaviour. The experimental setup consisted of two leaf discs of 2 cm of diameter connected by a small wooden bridge. Mites were placed in the first disc upon which mites were allowed to forage for 24h.

In the field this species was more abundant and in the laboratory it was a strong intraguild predator of *P. mexicanus*. *A. swirskii* was able to prey preferentially upon eggs of *P. mexicanus* than on its own, and to avoid patches colonised by the competitor. *P. mexicanus*, on the other hand, fed equally on its own eggs and on those of *A. swirskii*. *P. mexicanus* dispersal behaviour was quite chaotic, it did not correlate with risk of intraguild predation and it seemed that its strategy to find new prey patches was purely random walks (Figure 07). In the final experiment, Niry followed the dynamics of the two thrips in the presence of one mite, the other or the two of them together for 13 weeks. Generation times for the different species were between five and eight days, so the long-term dynamics reported on at least 10 generations of the different species. The results from this experiment revealed what we anticipated from previous observations. *A. swirskii* was

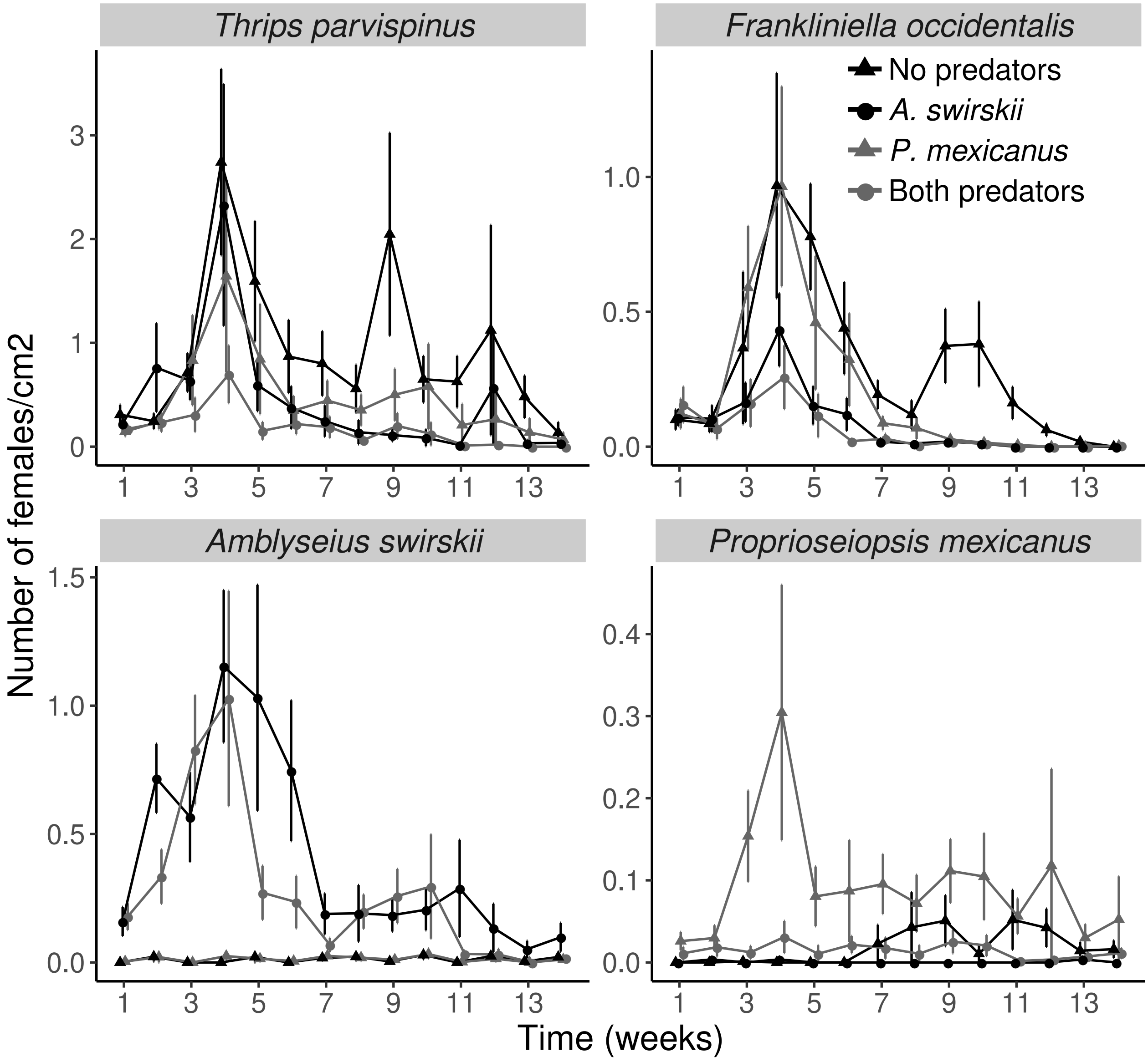

Figure 08. Population dynamics of the thrips *Thrips parvispinus* and *Frankliniella occidentalis* and their two predatory mites *Amblyseius swirskii* and *Proprioseiopsis mexicanus*. The different modalities include absence of predators, one species of predatory mite or both. The mean size of arthropod colonies (± SE) over the course of the experiment are presented (n=10). Mean size is measured as individual numbers per square centimetre of leave in a total of four leaves per replicate and week. Posthoc tests ($p < 0.05$) based on mixed effects models for arthropod numbers through time revealed that: *T. parvispinus* densities were significantly lower when both predators were present; *F. occidentalis* densities were larger in the absence of predators, *P. mexicanus* densities were lower in the presence of the superior competitor *A. swirskii.*

the best biocontrol agent, and thrips densities in the presence of *P. mexicanus* alone did not differ from densities in control cages without enemies. Adding *P. mexicanus* together with *A. swirskii*, however, significantly increased predation over herbivores relative to cages with *A. swirskii* alone, even if differences were small (Figure 08). In addition,

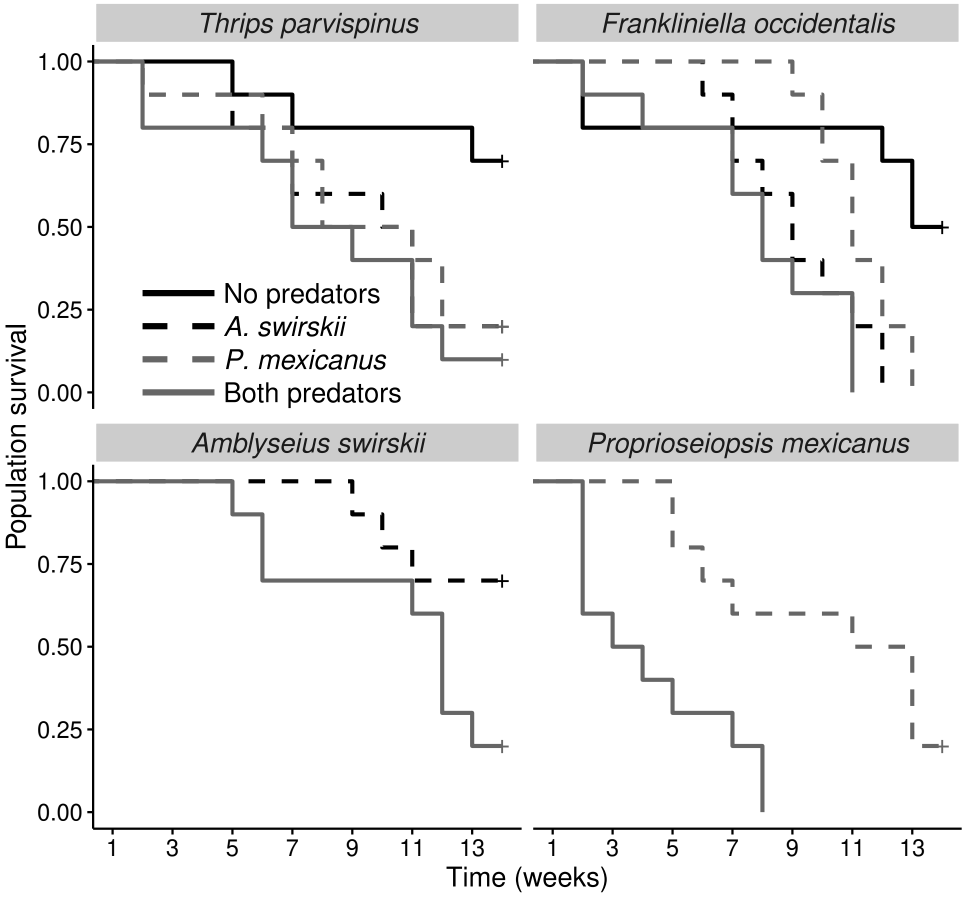

Figure 09. Persistence of the thrips *Thrips parvispinus* and *Frankliniella occidentalis* in the absence of predators, in the presence of one predatory mite or both (top), and persistence of the predatory mites *Amblyseius swirskii* and *Proprioseiopsis mexicanus* alone or in the presence of the other predator. The y-axis represents the proportion of microcosm cages in which each species survived. Posthoc tests ($p < 0.05$) based on survival analysis revealed that: both *T. parvispinus* and *F. occidentalis* survive for longer in the absence of predators; *A. swirskii* survival is not affected by the presence of *P. mexicanus*, but this later species becomes extinct faster in the presence of *A. swirskii.*

P. mexicanus suffered intense intraguild predation from *A. swirskii* and it became extinct in all cages after seven weeks (Figure 09). This yet unpublished study combining behavioural and ecological data from both the field and the laboratory, provided useful evidence of the little interest of *P. mexicanus* as a biocontrol agent. The ecological data accumulated also provided a behavioural understanding of why, even if both mites could eat each other, intraguild predation was far from symmetrical. Such asymmetry may be useful to explain why under some circumstances, some enemies suffering intraguild predation can become excluded from communities (van der Hammen et al., 2010; Montserrat et al., 2012; Fonseca et al., 2018; Marques et al., 2018). Predatory mites have been an important model for the study of intraguild predation and these little creatures have been found to show remarkable strategies to reduce such risk (Rasmy et al., 2004; Chow et al., 2010; Sato & Mochizuki, 2011; Van Maanen et al., 2012; Guo et al., 2016;

Guzman et al., 2016; Maleknia et al., 2016). Our study provided an unusual set of data revealing why a superior competitor can exclude another through a suite of advantageous behavioural traits including the capacity of avoiding risky patches, while displaying aggressive behaviours towards congeners. How *P. mexicanus* is able to avoid competitive exclusion in nature is a question that remains to be explored. Its apparently chaotic random walks could be the key. This type of dispersal strategy may allow some individuals to persist by locating prey patches that are free from superior competitors particularly in highly unpredictable environments where prey cues may be difficult to perceive, or when interspecific competition is strong (Benton & Bowler, 2012). It is thus likely that the poorer competitor *P. mexicanus* still wanders in Réunion greenhouses, but in 2023 in *La Coccinelle* website [8], out of the six natural enemies that can be purchased, the only mite that persists is *A. swirskii*.

2.5. INTRAGUILD PREDATION IN THE CONTEXT OF ENEMY DIVERSITY

The second PhD project I developed in Réunion was that of Karim Tighiouart. In his thesis Karim studied the effects of complex trophic interactions on the dynamics and stability of arthropod communities. Karim's PhD was supervised by Samuel Nibouche and by myself. In one of his experiments we increased the number of species that we manipulated in the previous PhD from four to eight, and the number of population cages from 40 to 80. This was one of the largest experiments I have ever been involved in and has set up the base of my main current project. We aimed at testing the relative influence of natural enemy diversity and intraguild predation in determining herbivore dynamics. More diverse assemblages of natural enemies have been often found to provide better biocontrol services. This positive effect is known as the biodiversity-biocontrol relationship (Letourneau et al., 2009; Snyder & Tylianakis, 2012; Dainese et al., 2019). The biodiversity-biocontrol relationship emerges through the “complementarity effect”. In complementary enemy assemblages, different species perform different functions, or partition their resources, for example by exploiting prey of different stages, or at different spatio-temporal scales (Pekas et al., 2016; Perović et al., 2018; Snyder, 2019). Complementarity reduces interspecific competition between enemies, facilitates natural enemy coexistence and may explain natural enemy diversity. From an applied perspective, complementarity can also ensure biocontrol services in the long-term. The positive effect of natural enemy diversity on biocontrol, however, can be dampened by “antagonistic effects” among enemies (Polis & Holt, 1992; Snyder & Tylianakis, 2012; Frago, 2016). In arthropod communities, these effects often occur due to intraguild predation. Despite decades of research on how enemy biodiversity and intraguild predation drive herbivore dynamics, experiments where the role of these two opposing forces has been tested together are still

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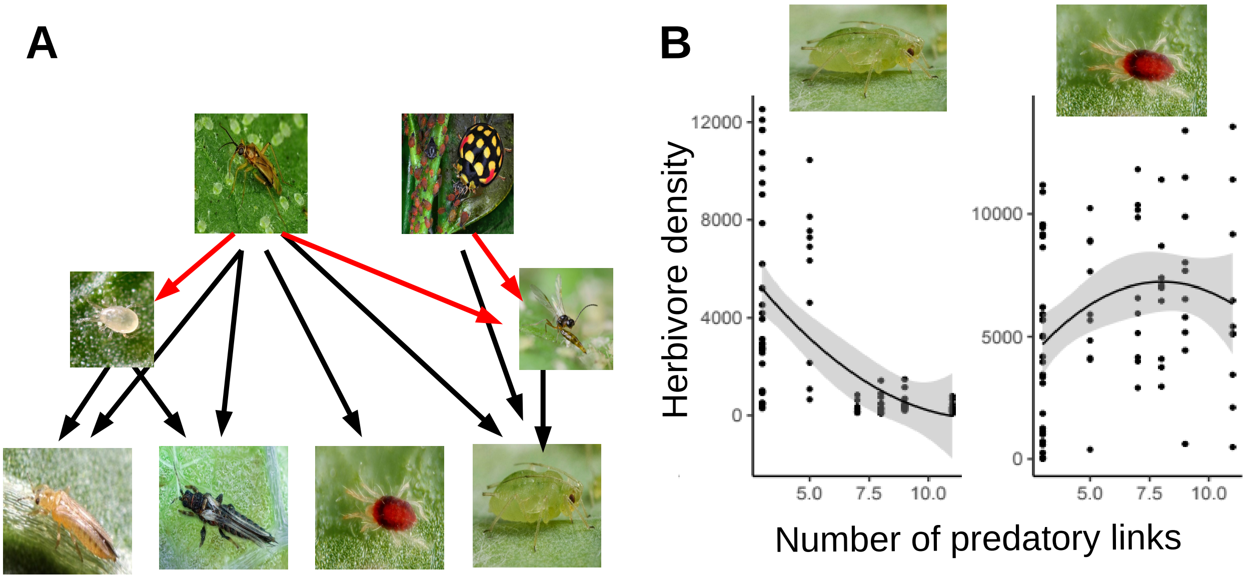


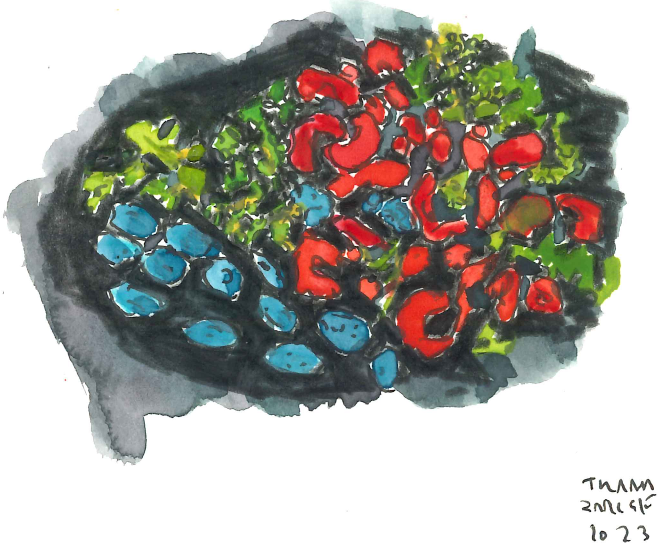
Figure 10. (A) The trophic web we manipulated in Karim Tighiouart's PhD comprising aphids, thrips and spider mites as herbivores and predatory mites, bugs, ladybirds and parasitic wasps as enemies. Arrows point from predator to prey. Red arrows represent intraguild predation links. (B) The relationship between predator links and herbivore numbers estimated as the sum of individuals per cage after 20 weeks of long-term dynamics. Based on mixed effects models for arthropod numbers through time the relationship was negative (i.e. stronger biocontrol) for the aphid *Myzus persicae*, but not for the spider mite *Tetranychus urticae* ($p < 0.05$). Photo credits by Antoine Franck.

very limited. During Karim's PhD we worked with a community of four herbivores including two thrips species (*Echinothrips americanus* and *Frankliniella occidentalis*), the spider-mite *T. urticae* and the aphid *Myzus persicae*. As natural enemies we took four of the six species available in *La Coccinelle* menu: one predatory mite *A. swirskii* (the superior competitor in the previous experiment), the ladybird *Cheilomenes sulphurea*, the parasitoid wasp *Aphidius colemani* and the generalist predatory bug *Nesidiocoris volucer*. The full community thus contained 11 predatory links, three of them intraguild (Figure 10-A), and was a replica of the pests and enemies that can be found in commercial greenhouses in Reunion. We studied the dynamics of eight different communities that varied in the number of links that were predatory (from 3 to 11) or intraguild (from 0 to 3). In each of the different communities we maintained the four herbivores and we manipulated the predatory layer by including two, three or four enemies. Just setting-up this experiment took a lot of organisation and one month of work. Karim inoculated plants with two consecutive waves of herbivores, followed by two waves of enemies. Twenty weeks of massive counting and adding plants followed, together with some cleaning up of enemy contaminations (some enemies were found in cages they were not supposed to). A lot of complex interactions likely took place during these 20 weeks of community dynamics, and the main results were the following. (i) Out of the four herbivores, the thrips *F. occidentalis* became extinct in all cages after seven weeks, likely due to both enemy pressure and strong interspecific competition for the plant resource. (ii) Intraguild predation links had a weak effect on herbivore dynamics. This supports theoretical work suggesting that this interaction does not necessarily reduce biocontrol in the long-term. The main reason for this result may be that by eating each other, predators can prevent overexploitation of prey, buffer prey extinctions, and prevent predator extinctions [reviewed in van Veen & Godfray (2012)]. (iii) In agreement with the biodiversity-biocontrol hypothesis (Letourneau et al., 2009), the densities of the thrips *E. americanus* and the aphid *M. persicae* were lower in communities with larger predation links. The density of the spider-mite *T. urticae,* however, showed an opposite pattern because its densities were significantly larger in communities with a larger number of predatory links (Figure 10-B). The interpretation of this result requires a close look at the full community we worked with. Relative to the spider-mite, the thrips and the aphid had a larger number of predators associated with them so it is likely that rich enemy assemblages suppressed spider-mite competitors thus releasing this species from interspecific competition (Morin, 2011).

(iv) By far, the most important element determining herbivore dynamics was the presence of the generalist predatory bug *N. volucer*. When this enemy was present the thrips *E. americanus* and the aphid *M. persicae* got strongly suppressed. This pattern is not unusual, according to Deborah K. Letourneau (Letourneau et al., 2009) when several species of natural enemies are present one predator species often dominates the other predators and takes over the control of herbivores. An experiment conducted by Straub & Snyder (2006) also showed a similar result, even if arthropod dynamics were followed during a sorter period of time. Their study revealed that comparing the effect of six species of natural enemies individually or grouped together, the biological control of aphids was not affected by predator richness but rather by the presence of particular natural enemies. In our study, the spider-mite *T. urticae* showed again a different and unexpected pattern because its densities were larger when *N. volucer* was present. As I mentioned above it is likely that this predator had a positive effect on the spider-mite by feeding on its herbivorous competitors. The beneficial effect of *N. volucer* on *T. urticae* is in agreement with Montoya et al. (2009) who suggested that as much as 40% of prey-enemy interactions may have a positive effect on prey through similar indirect positive effects (i.e. consumption of competitors). Behavioural interactions were likely to play an important role too because *N. volucer* is known to predate preferentially on aphids and thrips than on spider-mites (Marquereau et al., 2022). This experiment is not yet published, but the results have been presented by Karim or by myself at international conferences and invited seminars many times. Before publishing it, we aim at providing a deeper mechanistic understanding of the effects studied by coupling Lotka-Volterra theoretical models to our experimental data. More precisely, we aim at simulating different communities with four prey and four enemies with varying degrees of predatory and intraguild predation links, and enemy specialisation (to test the importance of generalist predators like *N. volucer*).

Altogether this study will be able to provide solid experimental evidence on how different enemies interact through complementarity or antagonism to determine herbivore dynamics and species extinctions. As far as I am aware, few experiments have manipulated a community as complex as the one that Karim worked with [but see for example Snyder et al. (2006) and Straub & Snyder (2006)]. Our experiment, however, revealed that working with such complex communities is a double-edged sword. Increased complexity is a better representation of natural systems, but finding general conclusions becomes more difficult. What holds for some species is not valid for others. Another limitation of this study is that our approach was to take a complex community and to make it simpler by removing species. It can be argued that what we observed can only apply to the particular eight species we worked with. The project that I started as coordinator in January 2023 aims at overcoming this limitation. For this we will collaborate with the biocontrol company *Biobest*, which has on the menu a set of natural enemies comprising almost 40 species.

CHAPTER 3: HERBIVORE SYMBIONTS DETERMINE INTERACTIONS WITH PLANTS AND NATURAL ENEMIES



Endosymbionts of the Asian citrus psyllid *Diaphorina citri* seen with the FISH technique
Carsonella in red, *Profftella* in green and host nuclei in blue
(watercolour on paper by tramabarcelo)

3.1. CONTEXT

Most terrestrial trophic webs can be depicted as tripartite representing plants, herbivores and higher-order consumers. This picture is now recognised as incomplete if microbial associates of these three different trophic levels are not taken into account because these microbes play key roles in the biology of most species (Antwis et al., 2020). This realisation would not have been possible without technological advances, and molecular biology in particular, that are making the study of microbes increasingly cheap and accessible to non-model organisms. I have been particularly interested in symbionts of insect herbivores, which are now recognised as key players in the dynamics and structure of insect trophic webs (McLean et al., 2016). I have been involved in the writing of several reviews on symbiont ecology, some of them in collaboration with PhD and MSc students. Most of these reviews were quite general and encompassed many different insect groups (Frago et al., 2012a, 2020; Monticelli et al., 2019; Zytynska et al., 2021; Frago & Zytynska, 2023). One of these reviews, however, focussed on symbionts of moths and butterflies (Paniagua Voirol et al., 2018) and was written in collaboration with Luis Paniagua-Voirol during his PhD in the Netherlands. During my second postdoc, Luis did his MSc in collaboration with Nina Fatouros and myself (Paniagua Voirol et al., 2020). He then started a PhD with Nina, but we kept collaborating with the writing of this review. My collaboration with Nina lasted even after I left the Netherlands and in 2020 (in collaboration with her and Sharon Zytynska) we wrote a book chapter entitled "Microbial symbionts of herbivorous species across the insect tree" (Frago et al., 2020). The aim of this review was to provide an order by order update of symbioses across herbivorous insects, particularly focusing on recent published evidence, and on how symbionts interact with plants and their defensive system. We showed that even if most insect orders require microbial services to be able to feed on plants, there are a multitude of strategies to do so (Figure 11). Sap-sucking feeders like aphids and whiteflies, for example, rely on obligatory associations, i.e. symbionts that are required for host survival. Plant phloem is of poor nutritional quality lacking many essential amino-acids that these groups of insects obtain from bacteria (Douglas, 1998). The diversification of sap-sucking groups could not be understood without these associations, which include *Buchnera aphidicola* in aphids, *Portiera aleyrodidarum* in whiteflies and *Carsonella ruddii* in psyllids (Dolling, 1991; Moran et al., 2008). These symbionts are more like an organelle than a symbiotic organism, with faithful transmission from mother to offspring and perfect congruence between symbiont and host phylogenies (Douglas, 1997). Other groups like phasmids, thrips and orthopterans mostly establish transient associations and/or rely on microbial genes that they have incorporated through horizontal gene transfer (Shelomi et al., 2016; Wybouw et al., 2016; McKenna et al., 2019). In holometabolous orders including lepidopterans, coleopterans and flies, symbionts need to persist through metamorphosis. Such transition often implies transient associations that are acquired from the environment at every generation (Moran et al., 2019), even if strict vertical transmission appears in

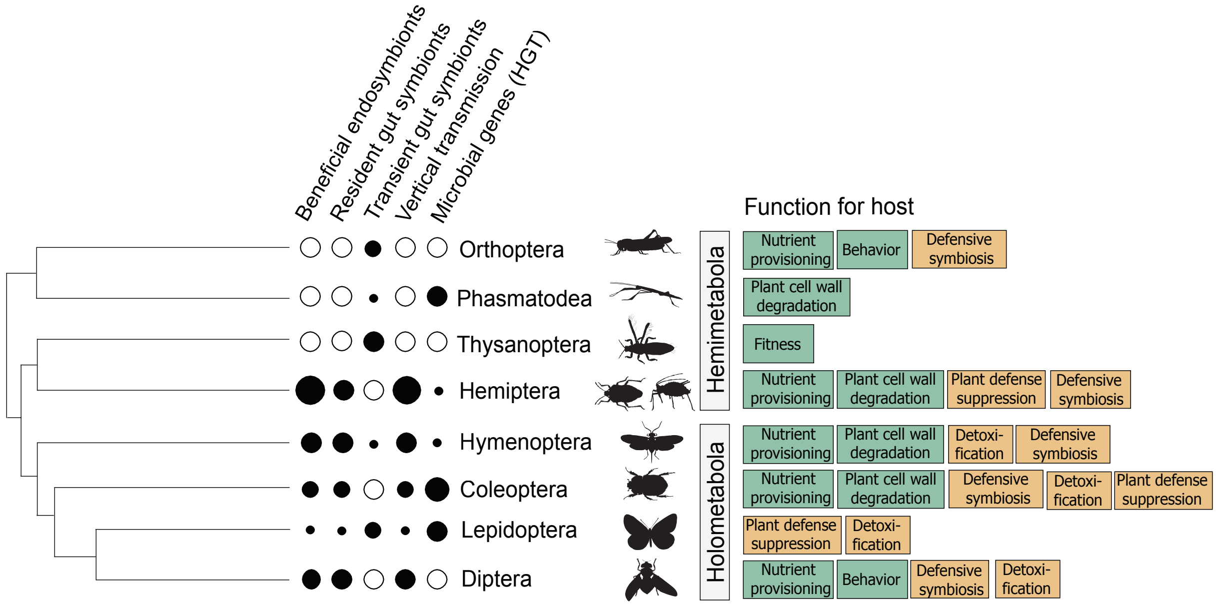


Figure 11. Different types of symbionts, transmission routes and functions for hosts across herbivorous insect orders. Phylogenetic tree was used from Misof et al. (2014). Herbivorous insects are distributed across four hemimetabolous and four holometabolous orders in total consisting of about 450,000 species (Wiens et al., 2015). Trait present in large amounts (large black circle) to small amounts (small black circle) to absent or not known/ studied (white circle). Green coloured means essential for the host, orange coloured means contribution to defences against natural enemies or plants. Modified from Frago et al (2020).

many species. In the book chapter we also provide examples of how insects co-opt microbial services to overcome the different lines of defences that plants employ to counter herbivore attack. Insect symbionts have long been known to aid in the detoxification of plant secondary metabolites. Some popular examples include gut microbes in desert locusts *Schistocerca gregaria* that degrade phenolic compounds (Dillon et al., 2000; Dillon & Charnley, 2002), the coffee berry borer beetle *Hypothenemus hampei* that carries a *Pseudomonas* bacteria in the gut that degrades toxic caffeine (Ceja-Navarro et al., 2015), or degradation of phenolic glycosides in the gut of the spongy moth *L. dispar* (Mason et al., 2014). Can symbionts help their hosts by impairing the induction of plant defences too? As I will explain in a dedicated section below, exciting discoveries from the last decade suggest so.

The last decades have also provided exciting discoveries on how symbionts may defend their hosts from natural enemies. The widespread use of defensive symbionts by animals can be appreciated in a review by Flórez et al. (2015). The authors provide examples including all major taxa, from sponges to vertebrates including molluscs, crustaceans and of course insects. Defensive symbionts have been found in many insect

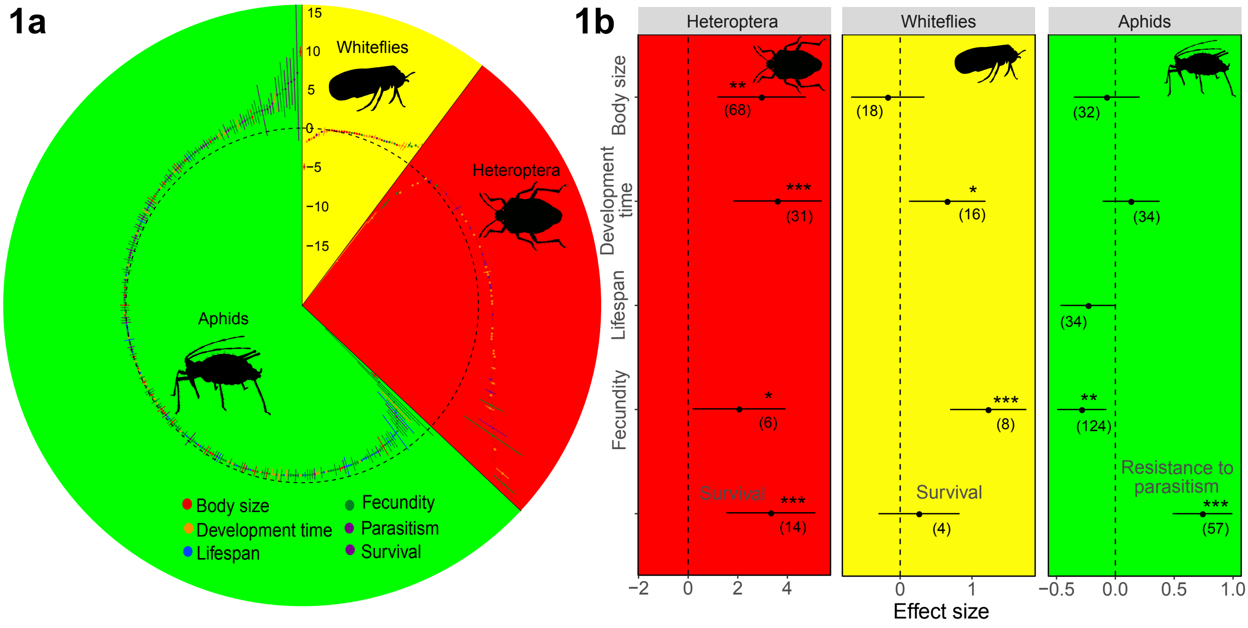


Figure 12. Overall effects of facultative symbionts on Heteroptera (Hemiptera: Heteroptera), Whitefly (Hemiptera: Aleyrodidae) and Aphids (Hemiptera: Aphididae). (a) circle plot shows all individual effect sizes extracted in our study (Hedges' g and 95% Confidence Interval) across the life history traits measured for the three taxon groups. (b) Overall effect sizes for the six main measured variables. Traits are considered a benefit (above zero) or cost (below zero) and significant when the 95% CI does not include zero. Body size includes size and mass traits, development time is measured in days for days until adulthood or first reproduction, lifespan is measured in days from birth or hatching until death, and fecundity is the number of offspring produced in a given time. Survival is measured as the proportion of individuals that survive to adulthood, and resistance to parasitism is measured as proportion survival after attack by a parasitoid wasp.*p < .05, **p < .01, ***p < .001. Modified from Zytynska et al. (2021).

groups. In the sawfly *Cephus cinctus*, for instance, the symbiont *Spiroplasma* encodes cardiolipin synthases and chitinases, which can poison sawfly enemies like parasitoids or parasitic nematodes (Ballinger & Perlman, 2019; Yeoman et al., 2019). In the bumblebee *Bombus terrestis*, gut symbionts reduced infection rates by the trypanosomatid parasite *Crithidia bombi* (Koch & Schmid-Hempel, 2011). In a recent example, Flórez et al. (2017) have found that in Lagriinae beetles, the extracellular bacteria *Burkholderia gladioli* protect eggs from pathogenic microbes. The symbiont is smeared onto the eggs during oviposition and by colonising the embryo and producing a cocktail of antibiotics it protects the eggs from pathogenic fungi. Probably one of the most well studied examples of an insect defensive symbiont is the aphid symbiont *Hamiltonella defensa*, a bacterium that protects its hosts from parasitoids likely through the production of toxins (Oliver et al., 2003, 2010). *H. defensa* is one of the many symbionts of aphids that is facultative. Facultative symbionts are not required for host survival but provide conditional benefits. *H. defensa*, for example, can be beneficial when natural enemies are present, but it becomes costly in their absence (e.g. Oliver et al., 2008; Simon et al., 2011; Vorburger & Gouskov, 2011). In collaboration with PhD student Karim Tighiouart and Sharon Zytynska we tackled this question by performing a meta-analysis (Zytynska et al., 2021). Symbionts were not at the core of Karim's thesis, but this study was an important side project that introduced Karim to meta-analytical techniques. We specifically explored the balance between costs and benefits of facultative symbiont infection in sap-sucking insects (i.e. aphids, whiteflies and true bugs). We found that aphids were the only group in which facultative symbionts imposed a fitness costs (i.e. reduced lifespan and fecundity), likely a trade-off emerging from some of these symbionts (like *H. defensa*) conferring resistance against parasitoids (Figure 12). Meta-analyses are useful to find general patterns from published literature, but also to identify areas where more research is needed. Despite the growing number of studies on insect symbionts, our analysis highlighted the need for more research in several areas and taxonomic groups (for example with whiteflies and non-model aphid species) to be able to make clear and general statements on the impact of insect symbionts on their hosts.

3.2. INSECT SYMBIONTS ALTER INDUCED PLANT DEFENCES AND PARASITOID FORAGING BEHAVIOUR

As discussed above, it has long been known that insect symbionts may aid their hosts detoxify toxic plant secondary metabolites that are constitutively present in plant tissues. Realisation that symbionts may aid at resisting induced defences, however, is more recent. By the end of my first postdoc in 2012 I was involved in the writing of a review paper where we provide examples suggesting that insect symbionts may manipulate plant physiology for the benefit of their host (Frago et al., 2012a). A classic example of such manipulation comes from the leaf-mining moth *Phyllonorycter blancardella*, which uses the bacterium *Wolbachia* to stimulate the production of the plant phytohormone cytokinin (Kaiser et al., 2010). These phytohormones prevent plant senescence and allow *P. blancardella* larvae to feed in autumn by creating a green island in an otherwise yellowing leaf. Removal of the symbiont eliminated the green island and increased larval mortality. In 2012, examples of mutualistic symbionts that manipulated plant physiology through downregulation of induced plant defences were rare and evidence was mostly indirect. In leaf-chewing insects, for example, elicitors of plant defences are often found in insect saliva and regurgitant (Bonaventure et al., 2011). A study by Spiteller et al. (2000) reported that some of these elicitors, in particular N-acylamino acids, were produced *in* *vitro* by symbionts isolated from moth larvae. More direct evidences reported symbiont involvement of downregulation of defence genes in the tomato psyllid, *Bactericerca cockerelli* (Casteel et al., 2012) and in the western corn rootworm *Diabrotica virgifera (Barr et al., 2010)*. This latter study, however, was later contradicted (Robert et al., 2013). Since these two studies did not report any measure of insect fitness, whether these interactions were truly mutualistic is a question that needs to be verified. Few years after these examples were published, clear evidence of symbiont manipulation of plant defences flourished (Mason et al., 2019). Our 2020 book chapter is a good example of these novel discoveries because it included three independent sections of symbiont modulation of induced plant defences in Hemiptera (aphids, whiteflies and psyllids), Coleoptera and Lepidoptera (Frago et al., 2020). As far as I am aware the first clear evidence of an insect-associated bacterium manipulating plant defences came from the Colorado potato *Leptinotarsa decemlineata* and subsequently from the whitefly *Bemisia tabaci (Su et al., 2015)*. These two examples were an important landmark, first because they reported that symbionts increased the fitness of the insect host through manipulation of induced plant defences. Second, because they provided solid evidence of the physiological mechanisms behind this effect. In both examples, insect saliva and regurgitant was used by insects to deliver symbionts or their products into the plant to trigger the induction of salicylic acid-dependent defensive pathways. As I explained before, the salicylic and jasmonic acid pathways crosstalk, so by triggering the former, the jasmonic pathway is downregulated. These changes thus had concomitant beneficial effects for the insect host because in both cases effective defences against the insect were based upon the jasmonic acid pathway. The use of symbionts to downregulate induced plant defences seems to be common in insects and has now been reported in many species. These include the use of the endosymbiont *Hamiltonella defensa* by the aphid *Sitobion miscanthi (Li et al., 2019)*, and gut microbes regurgitated to plant wounds in the false potato beetle *L. juncta (Wang et al., 2016)* and the Mexican bean beetle *Epilachna varivestis* (Gedling et al., 2018). In the two noctuid moths *Spodoptera frugiperda* (Acevedo et al., 2019) or *Helicoverpa zea* (Wang et al., 2017), however, symbiont benefits were less clear. In the *H. zea* example gut symbionts in oral secretion even betrayed their hosts by triggering defences that reduced moth fitness. Even if symbionts are not always helping their hosts, what is now clear is that insect interactions with plant defences need to take microbes into account. Most research has been done with bacteria, but important groups like fungi (Biedermann & Vega, 2020), protozoans or viruses are also likely to play important roles (Gurung et al., 2019). Most insects are colonised by a plethora of microbes and interactions between complex microbial communities are likely to determine host plant use by insects (Ferrari & Vavre, 2011). Ultimately the outcome of these interactions likely has cascading consequences for many species through the plant-mediated indirect effects we mentioned before.

In all these examples, symbionts aid their hosts withstand direct plant defences, which are those that poison or repel the herbivore. In a study that we performed using aphids as a model system, this defensive effect was found to extend to the manipulation of indirect plant defences, particularly parasitic wasp recruitment through the emission of plant volatiles (Frago et al., 2017). We performed this study during my second postdoc in the Netherlands and involved collaboration with two MSc students, Mukta Mala and Chenjiao Yang that I supervised. Mukta performed most of the behavioural analyses and by the end of her MSc she moved from the Netherlands to England (to the laboratory where I performed my first postdoc) to continue the experiments. Chenjiao did the tests on parasitoid attacks but once I already left the Netherlands so our interaction was mostly through weekly on-line meetings. In this study we found that the symbiont *H. defensa* rendered plants less attractive to the aphid parasitoid *Aphidius ervi* ultimately reducing attacks on young aphids. A metabolomic analysis of plant volatiles revealed that plants fed upon by aphids carrying or not the symbiont emitted the same molecules, but symbiont presence reduced their overall amounts. Based on this and previous studies, *H. defensa* thus stands as a symbiont that can manipulate plant defences upon aphid and whitefly feeding. Surprisingly, however, in our study we found that other aphid symbionts including *Regiella*, *Spiroplasma*, *Serratia* and *Rickettsiella* also manipulated the emission of plant volatiles and reduced plant attraction to the wasp. This result suggests that many symbionts, and not only *H. defensa,* may provide an indirect defensive service through manipulation of herbivore-induced plant volatiles.

An interesting question raised by the study I just presented is that defensive symbioses can protect their hosts against natural enemies in a more complex way than we previously thought. Most defensive symbioses act by improving host vigour, priming the host immune system, or by producing defensive chemical compounds (Brownlie & Johnson, 2009; Flórez et al., 2015). Many defensive symbioses, however, can act ahead of these types of defences by preventing natural enemies to locate and attack their hosts (Frago et al., 2020; Coolen et al., 2022). These examples motivated a review paper where we explored in collaboration with Sharon Zytynska the impact of herbivore symbionts on parasitoid foraging behaviour (Frago & Zytynska, 2023). We explored not only when symbionts impair host location and attack by parasitoids, but also when symbionts betray their hosts by producing cues that attract them (Figure 13). The examples that we provide were classified following the classic parasitoid literature that fractions the different steps that adult parasitoids need to take to parasitize a host. These steps include: detection of cues from the microhabitat, the host plant, direct and indirect host cues and host handling (Godfray, 1994; Vinson, 1998). We also provide evidence of how symbiont-related cues can be differently perceived by parasitoids depending on habitat diversity and the presence of parasitoid enemies including parasitoid competitors, hyperparasitoids and (again) intraguild predators. The importance of aphids in symbiont research was evident in this review as many examples came from this taxon. For example, in a recent report Goelen et al. (2020) isolated a bacterium from aphid honeydew that can potentially act as a defensive symbiont because it produces *in vitro* volatiles that repel parasitoids. In the pea aphid *A. pisum* the symbiont *Rickettsiella* increases the concentration of blue-green polycyclic quinones, which alters aphid colour from red to green (Tsuchida et al., 2010) making aphids less attractive to ladybirds (Libbrecht et al., 2007). If these types of defences fail, insects can still hope for reduced efficiency of natural enemies once handling

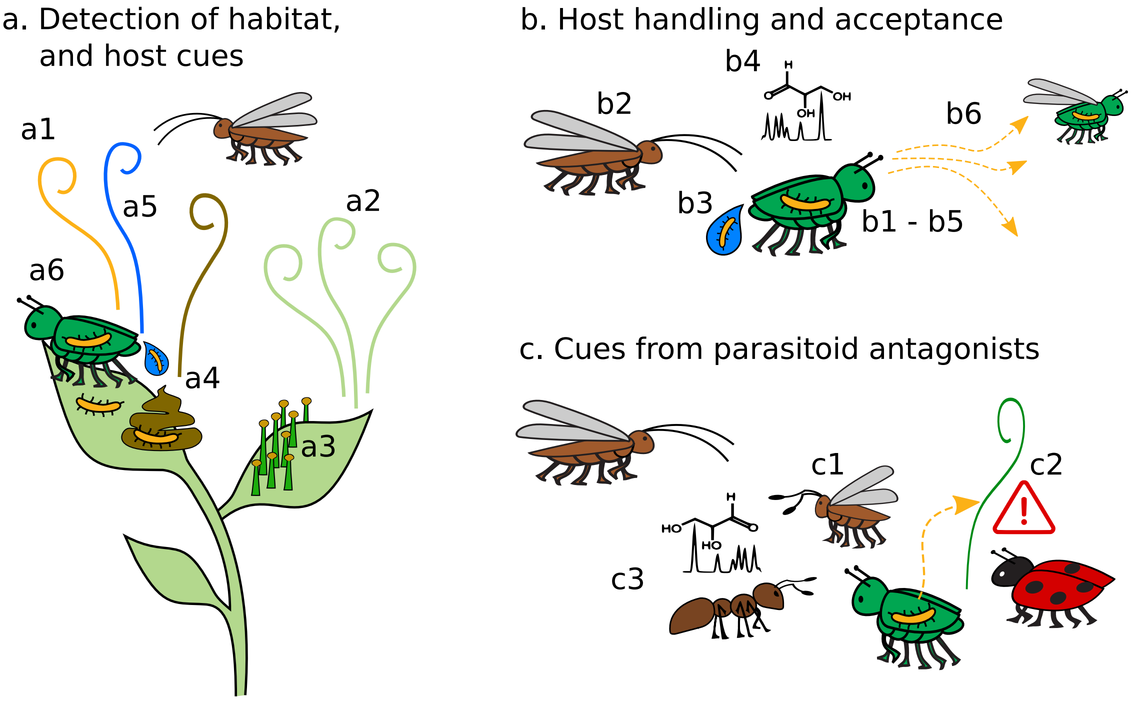


Figure 13. Herbivore symbiont effects on parasitoid foraging behaviour. Symbionts are represented by bacteria icons. a. Symbiont effects on cues from the microhabitat, the host plant, and the insect host. Parasitoids can detect volatile cues that emanate directly from symbionts (a1). Induced plant defences can be modulated by herbivore symbionts by reducing the emission of herbivore-induced plant volatiles (a2), or by altering plant physical structure (a3). Symbionts can alter indirect host cues emitted from frass (a4) or honeydew (a5), but also direct visual cues like insect body colour (a6). b. Symbionts effects on host handling and acceptance. By increasing growth rate or melanization, nutritional symbionts may reduce herbivores' window of vulnerability to parasitoids (b1). Parasitoids can detect insects defended by symbionts and adapt oviposition strategies (b2). Symbiont detection likely occurs through changes in honeydew composition (b3) or in cuticular hydrocarbon profiles (b4). Symbionts affect the defensive behaviours displayed by herbivores (b5), and how they disperse to avoid parasitism (b6). c. Symbionts alter risk cues associated with parasitoid antagonists. Symbionts modulate parasitoid competition (c1), and alarm pheromones emitted in response to intraguild predators (c2). Parasitoids evaluate patch quality based on the presence of antagonists like herbivore-defensive ants, which can alter symbiont hosting frequencies. Some parasitoids can deceive ants by emulating their cuticle hydrocarbon profiles (c3). Modified from Frago & Zytynska (2023).

prey. Many parasitoids, for instance, require hosts of a specific developmental stage or size (Godfray, 1994) and through nutritional services symbiont may defend their hosts simply by speeding-up juvenile development. This effect may reduce the time window prey are vulnerable to such enemies [e.g. (Belliure et al., 2008)]. A recent study reports a similar effect in nutritional symbionts of the saw-toothed grain beetle *Oryzaephilus surinamensis*, which is involved in speeding-up cuticle melanisation (Kanyile et al., 2022). The authors of this study found that by doing so handling time by spiders increased, which reduced successful attacks on the beetle. Relative to within-host defences, preventing parasitoid attacks may be a beneficial strategy likely to be less costly than constantly boosting the immune system. Reducing parasitoid attraction can also prevent the injury imposed by oviposition or auto-toxicity when bioactive molecules are involved [e.g. (Abram et al., 2019)]. This strategy, however, may be less efficient in complex habitats where parasitoids forage on a multitude of cues, which include not only attractive host cues, but also risk cues (like those derived from parasitoid antagonists) to be avoided (Frago, 2016).

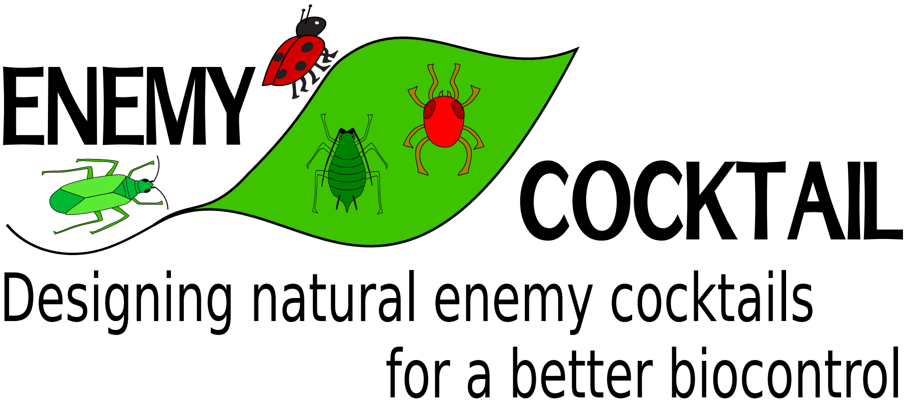
As I mentioned above, instead of defending their hosts, microbial associates of insects can also become the Achilles heel of the symbiotic association if they emit chemical traces that natural enemies use to detect their hosts (Frago et al., 2020; Coolen et al., 2022). This has been observed mostly in ectosymbioses, i.e. nutritional symbionts that live outside the host's body. Examples include parasitoid attraction to volatiles emitted from fungal and yeast symbionts of bark beetles (Boone et al., 2008), woodwasps (Faal et al., 2021) and *Drosophila* flies (Đurović et al., 2021), but also to beetle gut symbionts (Groba & Castelo, 2016). In these examples enemy attraction can impose a strong cost and potentially disrupt the symbiotic relationship. Evolution towards reduced enemy attraction, however, is likely to be constrained because the volatiles used by natural enemies are often metabolic by-products of the nutritional service provided by the symbiont (Davis et al., 2013; Groba & Castelo, 2016; Đurović et al., 2021). As our review showed, aphids have been a pivotal model to study insect symbiosis. More research on other taxa is needed, especially in groups like lepidopterans and coleopterans that carry very diverse symbiont assemblages in their guts (Moran et al., 2019). The study by Goelen et al. (2020) who identified a symbiont in honeydew that repelled parasitoids showed that we can exploit symbiont diversity from an applied point of view. Finding out culturable microbes of insect origin that are attractive to natural enemies could provide novel bioactive molecules to be used to manipulate enemy behaviour and to protect crops (Berasategui et al., 2016).

3.3. COMMUNITY-WIDE CONSEQUENCES OF DEFENSIVE SYMBIONTS

At the experimental level, I have performed a few experiments with the pea aphid *A. pisum* and the symbiont *H. defensa.* As an experimental ecologist I find this system very interesting from both a practical and a conceptual point of view. From a practical point of view the system is interesting because under long photoperiods (i.e. spring and summer-like conditions) most aphids reproduce asexually so we can keep aphid clonal lines in the laboratory (Dixon, 1977). Since most *A. pisum* facultative symbionts can be "cured" using antibiotics, we can establish clonal lines with or without symbionts in the laboratory and assess their effects on aphid biology with no influence of the aphid genetic background. From a conceptual point of view, the interest lies in the fact that symbiont defences are quite specific so that the conditional benefits that *H. defensa* provides are determined by the ecological context, and more in particular by natural enemy abundance and diversity (Vorburger, 2022). These defensive symbionts can thus be considered as defensive traits that can be acquired or lost and that we can easily manipulate. Research on defensive symbionts has tended to focus on their direct effects on the interaction between host and natural enemies (Oliver et al., 2010; Frago et al., 2020), and less on their far-reaching consequences at the community level. This lack of knowledge motivated a study in collaboration with Dirk Sanders where we explored how the symbiont *H. defensa* could trigger an extinction cascade by protecting its host from a specialist natural enemy (Sanders et al., 2016). We worked with an aphid–parasitoid community composed of three aphid species (*A. pisum*, *Aphis fabae* and *Megoura viciae*) and their associated specialist parasitic wasps (A. ervi, *Lysiphlebus fabarum* and *Aphidius megourae* respectively). Dirk showed in a previous study that this community was extremely stable through time because all six species were able to persist over many generations in population cages. The mechanism behind stability was shown to be parasitoid specialisation. The different enemy species specialise on different, potentially competing aphids thus reducing interspecific competition potential (Sanders et al., 2013). In this previous study Dirk and his collaborators showed that if a particular consumer species was lost or became rare, interspecific competition between prey species increased leading to their extinction, and as a consequence natural enemy species could also become lost, an extinction cascade (Sanders et al., 2013, 2015). In our symbiont study we included into the system the defensive symbiont *H. defensa* in the aphid *A. pisum*. The symbiont released its host from top-down pressure so that this aphid dominated the community making the other two species rare and their associated parasitoids to go extinct (Figure 14). Diversity is usually good for ecological stability (Cardinale et al., 2012; Tilman et al., 2014; Liang et al., 2016, 2022; Emmett Duffy et al., 2017; Hagan et al., 2021; Wagg et al., 2022) and our experiment revealed how important insect symbionts can be for community stability and ultimately species persistence and diversity.

The year 2023 marks the 20th anniversary of the discovery of the defensive role of *H. defensa (Oliver et al., 2003).* Despite two decades of intense research with this symbiont, many questions are still unanswered. Does this symbiont provide services that are yet to be discovered? How do complex community interactions determine symbiont prevalence in aphid populations? How do symbiont presence feed-back to the dynamics of complex plant-herbivore-natural enemy communities? As I have explained above, and will explain in the following section, some of my experimental research aims at filling these gaps, but most of my experiments have provided more new questions than answering old ones. All these experiments revealed that like most insects, aphids are found "between the devil and the deep blue sea" (Lawton & McNeill, 1979), but their fight against plant defences or natural enemies could not be understood without considering versatile symbionts like *H. defensa.*

CHAPTER 4: RESEARCH PROJECT



Logo of the EnemyCocktail project that will run from 2023 to 2026

4.1. DESIGNING NATURAL ENEMY COCKTAILS FOR A BETTER BIOCONTROL

a. Context

In January 2023 I started the EnemyCocktail project "Designing natural enemy cocktails for a better biocontrol" as coordinator. This project is funded by the French *Agence National de la Recherche* and it will last until 2026. The main idea behind this project is to take a community ecology approach to improve inundative biocontrol [i.e. based on natural enemy mass rearing and release (Hajek & Eilenberg, 2018; Stenberg et al., 2021)] using natural enemy combinations, or cocktails. This project is a collective effort of scientists and biocontrol practitioners from France, England and Belgium. We will also collaborate with the company *Biobest* who mass produces enemies for release in commercial greenhouses. An important constraint for the success of biocontrol is that it relies on deep ecological knowledge of species interactions and, as I have already shown in previous pages, its success is often hampered by the intricate nature of ecological interactions. An important challenge in biocontrol is to find the best natural enemy cocktails to control pests by minimising antagonistic interactions between them (Shakya et al., 2010; Messelink et al., 2014). This is particularly relevant in inundative biocontrol because mass releases concentrate enemies and promote interactions between species, but also because single enemy releases are seldom enough to control pests (Messelink et al., 2014). Ecological knowledge in this context is key for the success of a biocontrol program because either in greenhouses or in natural ecosystems natural enemy impacts on prey can rarely be predicted from pairwise species interactions (Sih et al., 1998; Veen et al., 2005; Ives & Carpenter, 2007; Estes et al., 2011; Hajek & Eilenberg, 2018).

The project will use ecological theory on the relationship between natural enemy diversity and biocontrol, i.e. the biodiversity-biocontrol relationship (Snyder & Tylianakis, 2012; Dainese et al., 2019). This relationship emerges through the “complementarity effect”, which can be dampened by "antagonistic effects" like intraguild predation. These effects have been largely discussed already. A new element, however, is the fact that we will manipulate complementarity and antagonism in experiments based on natural enemy functional traits. Enemy assemblages that are functionally diverse differ at the trait level and therefore perform more variable functions (Pekas et al., 2016; Perović et al., 2018; Snyder, 2019). Functional traits (often simply called "species traits") are increasingly considered as fundamental to understanding species interactions because they are based on species phenotypes and go beyond simple lists of species (Dawson et al., 2021). Functionally diverse assemblages are considered more complementary because they partition their resource use (Barry et al., 2019). These assemblages thus exploit prey of different stages or at different spatio-temporal scales, or they display different hunting styles (e.g. passive vs active hunters) (Snyder & Tylianakis, 2012; Pekas et al., 2016; Perović et al., 2018; Snyder, 2019).

Despite testable predictions on the role of complementarity and intraguild predation on the biodiversity-biocontrol relationship (Polis & Holt, 1992; Snyder & Tylianakis, 2012; Pekas et al., 2016; Perović et al., 2018; Dainese et al., 2019; Snyder, 2019), conclusive evidence that tests the relative role of these two opposing mechanisms is lacking. We particularly lack information that pinpoints how these two mechanisms operate in combination to drive the dynamics of multi-predator communities, a knowledge needed to efficiently implement biocontrol. Separating these two opposing forces is important because in applied biocontrol many enemies are generalists and often act as intraguild predators by feeding on herbivores but also on other predators (Welsh et al., 2012). Most previous research has focussed on a given model community with a limited number of natural enemies involved (Vance-chalcraft et al., 2007), which limits our ability to infer general patterns. We lack experiments with real replicates at the community level. It is thus important to work with an experimental system able to bring theory and empirical evidence together to get a predictive understanding of the effect of predator assemblages on herbivore dynamics. Studies that explore the dynamics of communities over multiple generations are also needed to understand the conditions leading to lasting biocontrol, and the long-term persistence of natural enemies (Turchin & Taylor, 1992; Bonsall & Hassell, 1997; Veen et al., 2005; Frago & Godfray, 2013; Sanders et al., 2013, 2015). Few studies have also worked in semi-natural conditions to assess how mass released enemies engineer pre-existing trophic webs, even if this question has been considered when exotic enemies are released to control invasive pests (Willis & Memmott, 2005; Todd et al., 2021). The EnemyCocktail project will aim at filling some of these gaps through a meta-analysis, the development of experiments at different spatial scales (from population cages in the laboratory to commercial greenhouses) and theoretical modelling. The overarching aim of the project is to answer key fundamental questions with a precise applied objective. At the fundamental level we will tackle questions on how natural enemies drive the dynamics of herbivores and how these interactions drive species persistence and modulate extinctions. At the applied level, our ultimate goal is to design natural enemy cocktails to test them in commercial greenhouses and to lay the ground for their commercial implementation. This will address a societal demand by advancing in the field of agroecology thus making the techniques and end products available to a larger public (Hulot & Hiller, 2021). This will ultimately allow proposing a roadmap for the selection of natural enemy combinations and will extend biocontrol to crops where they are not yet used.

b. Project objectives

For the experimental and modelling part of the project we will work with two important pests of cucumber and sweet-pepper: the two-spotted spider mite *T. urticae* and the green peach aphid *M. persicae* (Dedryver et al., 2010; Meck et al., 2013). As natural enemies, we will use those mass produced by our industrial partner *Biobest.* The project is organised in three Objectives: (i) To test the hypothesis that natural enemy communities with strong complementarity and weak intraguild predation provide better long-term biocontrol services. (ii) To use theoretical models for understanding the combined effect of complementarity and intraguild predation on biocontrol outcomes. (iii) To evaluate the efficiency of natural enemy cocktails in cucumber *Cucumis sativus* and sweet-pepper *Capsicum annuum* commercial greenhouses.

Objective 1a - The meta-analysis. We will develop the first objective with a PhD student that will be recruited in September 2023 and in collaboration with CBGP partners (Philipp Auger, Denise Navia, Marie-Stéphane Tixier and Anne Xuéreb), Biobest France and Belgium (Azélie Lelong and Tolis Pekas) and our English collaborator (Dirk Sanders). This objective has two different projects, the first one is to develop a meta-analysis to test whether arthropod biocontrol is stronger when enemies are more complementary and when they engage in low intraguild predation. Fifteen years ago Vance-chalcraft et al. (2007) already performed a similar meta-analysis but considering exclusively intraguild predation. We will create a novel database by adding more recent data, and we will also add to the analyses natural enemy complementarity. We will obtain this latter information from published literature based on species traits and by calculating functional niche differentiation using dedicated multivariate techniques (Laliberté & Legendre, 2010; Dawson et al., 2021). As done in previous trait databases for birds and plants (Kattge et al., 2020; Tobias et al., 2022) our database will include "the characteristics of organisms that determine its performance in response to the environment and/or its effects on ecosystem functioning" (Dawson et al., 2021). These will include feeding guild (predator vs parasitic wasp), taxon (hoverfly, lacewing, ladybird, parasitic wasp, etc), predatory strategy (i.e. active vs passive hunters), and several life-history traits like size, fecundity, dispersal capability or development time. We will also obtain potential for intraguild predation between enemies. Following Vance-chalcraft et al. (2007) we will classify intraguild predation as absent, symmetrical or asymmetrical. As I have already explained, symmetrical intraguild predation occurs when both predators can feed on each other, whereas asymmetrical intraguild predation occurs when one predator (the intraguild predator) can feed on the other (the intraguild prey) but not vice-versa (Polis & Holt, 1992; Frago, 2016).

Objective 1b - Population cage experiments. This part will be the core of the thesis that will start in September 2023. We will test experimentally the biocontrol potential of natural enemy cocktails that vary in their degree of complementarity and intraguild predation strength on the green peach aphid *M. persicae* feeding on sweet-pepper *C. annuum* and on the two-spotted spider mite *T. urticae* feeding on cucumber *C. sativus*. For the experiments we will take a two-by-two factorial design crossing these two factors by selecting pairs of enemies from the list of species available from *Biobest* [9](Figure 14).

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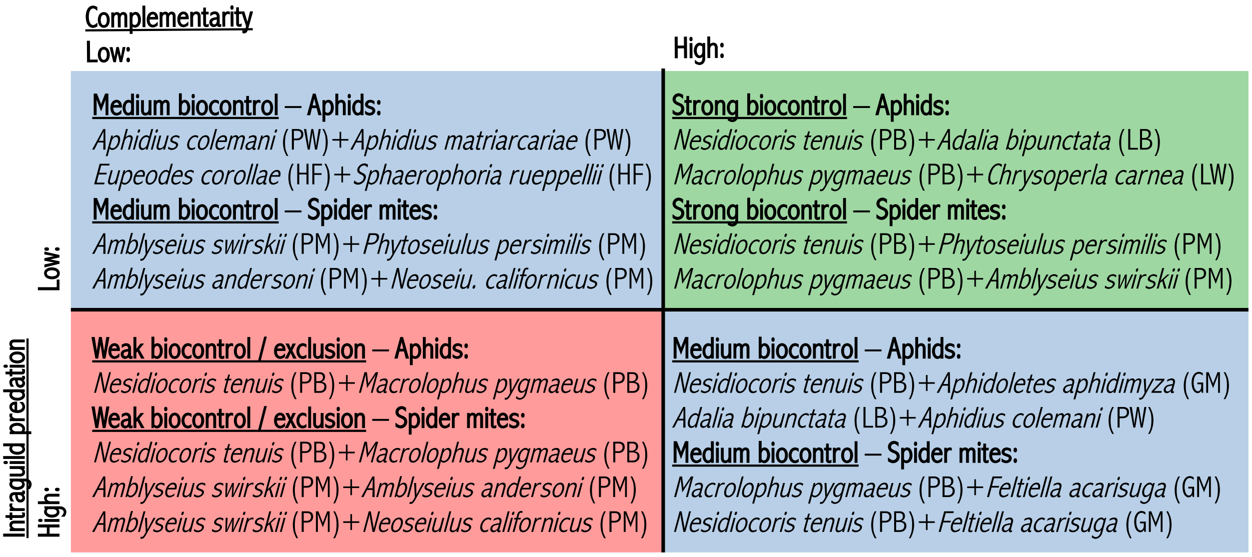
[9] 9-https://www.biobestgroup.com/en/biobest/products/biological-pest-control-4463/#productGroup_4479



Intraguild predation	Complementarity Low:	High:
Low:	**Medium biocontrol** – Aphids: *Aphidius colemani* (PW)+*Aphidius matriarcariae* (PW) *Eupeodes corollae* (HF)+*Sphaerophoria rueppellii* (HF) **Medium biocontrol** – Spider mites: *Amblyseius swirskii* (PM)+*Phytoseiulus persimilis* (PM) *Amblyseius andersoni* (PM)+*Neoseiu. californicus* (PM)	**Strong biocontrol** – Aphids: *Nesidiocoris tenuis* (PB)+*Adalia bipunctata* (LB) *Macrolophus pygmaeus* (PB)+*Chrysoperla carnea* (LW) **Strong biocontrol** – Spider mites: *Nesidiocoris tenuis* (PB)+*Phytoseiulus persimilis* (PM) *Macrolophus pygmaeus* (PB)+*Amblyseius swirskii* (PM)
High:	**Weak biocontrol / exclusion** – Aphids: *Nesidiocoris tenuis* (PB)+*Macrolophus pygmaeus* (PB) **Weak biocontrol / exclusion** – Spider mites: *Nesidiocoris tenuis* (PB)+*Macrolophus pygmaeus* (PB) *Amblyseius swirskii* (PM)+*Amblyseius andersoni* (PM) *Amblyseius swirskii* (PM)+*Neoseiulus californicus* (PM)	**Medium biocontrol** – Aphids: *Nesidiocoris tenuis* (PB)+*Aphidoletes aphidimyza* (GM) *Adalia bipunctata* (LB)+*Aphidius colemani* (PW) **Medium biocontrol** – Spider mites: *Macrolophus pygmaeus* (PB)+*Feltiella acarisuga* (GM) *Nesidiocoris tenuis* (PB)+*Feltiella acarisuga* (GM)

Figure 14. Expected biocontrol outcomes based on low and high natural enemy complementarity and intraguild predation. Details on the natural enemy pairs/cocktails that we will use are also shown. Enemies include parasitic wasps (PW), hoverflies (HF), predatory mites (PM), predatory bugs (PB), ladybirds (LB), lacewings (LW) and gall midges (GM).

We will manipulate complementarity by selecting natural enemies based on functional niche differentiation as explained above (Laliberté & Legendre, 2010). We will also estimate antagonism from the literature, but also from the knowledge that our collaborators at *Biobest* have. Not surprisingly, the approach that we will take will imply population cages and following long-term community dynamics. This will allow us to assess if some enemy combinations are unstable and some enemies exclude others, an important element because enemy persistence is synonymous with lasting biocontrol services (Hajek & Eilenberg, 2018). The extinction of some enemies can inform about species pairs that are not compatible, ultimately helping us to design the best enemy cocktails. In addition, long-term experiments will provide precious data to feed the theoretical models that I will explain below. When describing the experiment that we performed in the PhD of Karim Tighiouart I mentioned that one limitation of the study was that our approach was to take a complex community and to make it simpler by removing species. This implied that the same species were often present in the different community types. The experiment proposed here will allow us to test the effect of multiple enemy assemblages using a multitude of different natural enemies. This approach will allow us to have "real" replicated communities. In addition we will test similar hypotheses using two herbivores, an aphid and a spider-mite. All in all, this experiment will allow us to propose more general conclusions than in many other previous studies.

Objective 2 - Theoretical models. I will perform this objective in collaboration with Vasilis Dakos and a postdoc that we will recruit at ISEM (The Institute of Evolutionary Science of Montpellier). In this objective we will use models to understand the combined effect of complementarity and intraguild predation on biocontrol outcomes. The overall aim of this Objective is to understand if our theoretical expectations agree with the experimental outcomes in population cage experiments. More importantly, we will use the outcomes of these models combined with experimental data to identify the best natural enemy cocktails for biocontrol that we will test in the field. We will take two different approaches. First, (i) we will analyse mechanistic stage-structured population models that describe the experimental setups of the experiments, and second (ii) we will infer the relative effect of complementarity and intraguild predation as well as the effect of natural enemies on pest biocontrol. In the first approach we will take advantage of the long history of theoretical population models developed for the study of trophic interactions with intraguild predation. These models have been often used to understanding the dynamics of predators competing for a shared resource [e.g. Polis & Holt (1992) and McEvoy (2018)] Theoretically, intraguild predation usually limits coexistence of the natural enemies leading to competitive exclusion (Verdy & Amarasekare, 2010) and inevitably pest control could become inefficient (Denoth et al., 2002b). At the same time, various mechanisms have been modelled to increase stable coexistence between the competing natural enemies on the common pest resource (Amarasekare, 2008). For instance, temporal or spatial aggregation (Murdoch & Briggs, 1996), or differences in the attack rates of the two competing predators (Mills & Getz, 1996). These effects could increase complementarity, allow for resource partitioning thus relaxing competition. Models combining intraguild predation with different complementarity mechanisms, however, have been much less explored. The stage-structured population models (Murdoch et al., 2013) that we will develop are directly linked to the experimental communities and will be parametrized when possible with the experimental data from population cage experiments. The models will allow us to explore if our empirical observations will match our theoretical expectations for the coexistence of natural enemies and for pest control based on the mechanisms of intraguild predation and complementarity. In addition, we will use them to identify the conditions under which complementarity and intraguild predation when acting together could maximise biocontrol. Recently, it was theoretically shown that combining pathogen-pest and parasitoid-pest interactions that separately lead to unstable pest control dynamics could enable coexistence and rescue pest control (Ong & Vandermeer, 2015). We expect that our population models will provide similar insights on pest dynamics.

The second approach will involve inferring the relative effect of complementarity and intraguild predation as well as the effect of natural enemies on pest biocontrol using Empirical Dynamical Modelling (Sugihara et al., 2012; Munch et al., 2020). These models are more modern and do not assume a specific community structure, but allow inferring interactions between species directly from the observed time series. This approach has been used to identify causal relationships between interacting climatic variables (van Nes et al., 2015), but also interaction strengths between fish species in a lake (Ushio et al., 2018). We will use these models to identify the net effect that is the sum of all direct and indirect interactions between two species averaged through time (Deyle et al., 2016). For example, in a 40 species insect community this approach recently revealed species effects on each other to be attributed to indirect and possible non-trophic interactions that are otherwise hard to estimate (Kawatsu et al., 2021). In our case, we focus on estimating, first, the net effect of each natural enemy on the other as a proxy of the relative strength of complementarity and intraguild predation mechanisms, and second, the net effect of each natural enemy on the pest as a proxy of biocontrol efficiency.

Objective 3 - Experiments in commercial greenhouses. We will develop this Objective once laboratory experiments and theoretical modelling are finished. With the data obtained we expect that we will be able to select candidate cocktails to be tested in commercial greenhouses. Simply put, we will scale-up what we observed in 40x40x40 cm cages to greenhouses. We will develop this Objective in tight collaboration with *Biobest* in Belgium who will mass produce the enemies, but also with *Biobest* France that will aid us in selecting the greenhouses where we can deploy the enemies. The main objective will be to test the biocontrol potential of natural enemies on green peach aphid *M. persicae* and on two-spotted spider mite *T. urticae* in sweet-pepper and cucumber greenhouses. The approaches taken in Objectives 1 and 2 are important first steps to understand predator-prey interactions, even if in population cages dispersal is not possible due to the lack of spatial structure and theoretical models lack refinement in the biology of the interactions taking place. Enemy cocktails thus need to be validated directly in greenhouses to ensure their success in biocontrol. From a fundamental point of view, greenhouses commonly harbour diverse networks of pests and associated enemies (Snyder & Tylianakis, 2012; Messelink et al., 2014; Snyder, 2019; Expert Group for Technical Advice on Organic Production, 2022), so that inundative biocontrol represents a unique opportunity to experimentally assess how new enemies integrate and modulate natural ecological networks [e.g. (Willis & Memmott, 2005)]. For example, antagonistic interactions observed in population cages are likely to be relaxed in greenhouses because the spatial complexity of a greenhouse may provide refuges to herbivores or may allow enemies to avoid intraguild predation (Tylianakis & Romo, 2010). Natural enemy releases can also trigger outbreaks of secondary pests (Willis & Memmott, 2005; Hajek & Eilenberg, 2018), for example if the density of the targeted pest is reduced with concomitant positive effects on other herbivores that are released from intraspecific competition (Tylianakis & Romo, 2010). Arguably, performing experiments in larger scales like open orchards would represent an even more realistic scenario, but in these scenarios enemies would disperse and become too diluted to monitor their dynamics or their impact on pests [but see (Willis & Memmott, 2005)]. We will perform the experiments in replicated commercial greenhouses infested with either *M. persicae* aphids or *T. urticae* spider mites. To validate the results from previous Objectives we will compare for each pest species two different enemy cocktails that differ in their biocontrol efficiency: one with strong and one with weak expected biocontrol potential. We will also compare the efficiency of these two cocktails with releases of single enemies. We will assess cocktail efficiency at two different levels: on predation and parasitism on targeted pests *M. persicae* and *T. urticae*, but also on secondary pests. To achieve this we will estimate the abundance of the different herbivores found in the greenhouses and that of their associated enemies. By comparing abundances before and after enemy releases we will assess parasitism and predation on targeted pests, but also changes in the abundance of other herbivores likely to become secondary pests. By the end of the production season we will measure plant yield to assess biocontrol services on crop protection. We will combine data on pest abundance and yield to investigate whether successful biocontrol on targeted pests precludes crop protection due to outbreaks of secondary pests.

c. Project implications

Biocontrol is emerging as a key technique for the control of arthropod pests, but its efficient implementation is often challenging because it needs deep ecological knowledge of species interactions (Hajek & Eilenberg, 2018). Enemy combinations are currently used in inundative biocontrol but we are still far from an efficient use of these cocktails (Lamichhane et al., 2017; Stenberg, 2017; Barratt et al., 2018; Noriega et al., 2018; Hulot & Hiller, 2021). We will provide solid empirical evidence to solve this scientific challenge with important applied consequences. There is a long-standing open question about whether intraguild predation challenges pest control (Polis & Holt, 1992; Denoth et al., 2002b; Morin, 2011; Messelink et al., 2014; Frago, 2016), and by using highly replicated experiments coupled with theoretical models and field tests, we will unveil the conditions under which this interaction is clearly antagonistic. Finding out that some combinations with strong intraguild predation in fact allow the long-term persistence of enemies and thus of biocontrol services, could reverse a paradigm in community ecology.

Herbivorous arthropods are among the most diverse animals in terrestrial ecosystems,and their diversity is only comparable to that of their associated natural enemies (Stork, 2018). Understanding the mechanisms that drive the dynamics of these communities goes far beyond applied biocontrol. In natural ecosystems, natural enemies suppress herbivores and by doing so they indirectly modulate plant productivity (White, 2005) and biogeochemical cycles like those dependent on leaf decomposition and soil mineralisation (Tamura et al., 2017). This project will also help to understand the forces that maintain diversity. Finding out that predators that eat each other often engage in a positive interaction that stimulates their persistence would be an exciting "proof of concept" discovery. In view of current biodiversity declines, understanding positive interactions allowing the persistence of higher trophic levels is a key contemporary question of broad, fundamental interest (Borrvall et al., 2000; Mooney et al., 2010; Kehoe et al., 2020).

Far from the study of community ecology and biocontrol, this project has important economic and societal implications. Agriculture in the European Union will face serious challenges in the near future, which include lack of water resources, climate change, reduced competitiveness and increased costs. Production in greenhouses is increasingly seen as a solution to some of these issues. In 2009, greenhouse production was about 800 000 ha globally, and 20% (160 000 ha) was located in Europe (Expert Group for Technical Advice on Organic Production, 2022), which shows the economic importance of this sector in our region. Aphids (Dedryver et al., 2010) and spider mites (Meck et al., 2013) are two of the most important pests in greenhouses causing huge economic losses yearly due to direct effects on plants or the transmission of pathogens. We will provide specific guidelines to combat these two pests, a direct economic impact, but also a more general roadmap for the selection and efficient use of natural enemy cocktails in inundative biocontrol. This is a needed tool because most enemy combinations used in biocontrol are not selected based on solid scientific evidence. For example, through the lens of community ecology we will be able to document failure of inundative biocontrol due to the outbreak of secondary pests. By proposing clear guidelines on the enemy combinations to be used, we will render these cocktails more efficient and consequently cheaper. More importantly, efficient and cheaper biocontrol techniques will increase confidence in these techniques and extend them to a larger range of farmers.

At the societal level, our project will advance in the use of biocontrol, which is probably the best alternative to control pests without insecticides. Biocontrol is an important ecosystem service delivered by biodiversity that is key for the transition to agroecological production. The transition to agroecology will reduce dependency on chemical pesticides with concomitant effects on consumer and farmer health, and protect natural fauna. The project will also open the door to new projects implementing novel biocontrol techniques at a larger scale or the production of new commercially-available products. As in many disciplines, ecology and entomology are fields with a dramatic gender gap whereby women are under-represented in higher degree stages (i.e. permanent positions and positions of prestige and power) [e.g. (Walker, 2018; Hipólito et al., 2021)]. In an effort to reverse this situation, in our project we have four women out of nine partners, two of them as leaders in Partner institutions.

4.2. PLANT-THRIPS-SYMBIONT INTERACTION WEBS

My experimental work with insect symbionts currently focuses on thrips (Thysanoptera). Thrips are an order of slender insects, of approximately 1 to 3 mm long with about 6.000 extant species described worldwide (Stork, 2018). Thrips larvae can be herbivorous, predatory or fungivorous and many species disperse to flowers as adults to feed on pollen or to mate (Mound, 2005). My work with thrips started during Niry Dianzinga's PhD in Reunion. We used this group of insects to study insect diversity along environmental gradients by sampling them from flowers (Figure 15). Elevational gradients are useful to study how abiotic conditions shape communities. Along elevational gradients, assemblages of species originating from the same species pool but which are found at very different conditions can be studied within small geographic distances (Hodkinson, 2005; Sundqvist et al., 2013; Tylianakis & Morris, 2017). The environmental changes occurring through elevation also impact plant communities so elevational gradients are also useful to study changes in plant-insect interactions (Kreft & Jetz, 2007). In Niry's study we sampled insects along seven replicated elevational gradients in winter and summer. We obtained a total of 4,280 specimens comprising 40 species from flowers of 106 different plant species. With this observational data we reported how different diversity metrics varied with elevation but also due to environmental variables and landscape features (Dianzinga et al., 2020). We took a similar approach during a sampling expedition to Madagascar. Thanks to these two sampling campaigns we discovered two new species: *Thrips reunionensis* from Reunion (Goldarazena et al., 2020) and *Dendrothripoides moundi* from Madagascar (Goldarazena et al., 2020).

During Niry's study we did not focus on thrips symbionts, a research line that I started in 2020 in collaboration with Emmanuelle Jousselin at CBGP. We got funding from CeMEB (Centre Méditerranéen Environnement et Biodiversité) to develop an exploratory project on thrips symbionts. The project was exploratory because knowledge on thrips microbial symbionts is so far very limited (Frago et al., 2020). Symbionts in thrips had been investigated only in a few species, mainly pests. Some herbivorous thrips harbour gut bacteria, consisting largely of Enterobacteriaceae. Schausberger (2018) presented an overview of the endosymbiotic and gut/saliva bacteria from the thrips family Thripidae, which contains most pest species. For example, two of the most widespread pest species, the western flower thrips *F. occidentalis* and the onion thrips *Thrips tabaci* both associate with γ-proteobacteria, including *Erwinia* and *Pantoea* or *Pantoea*-like bacteria. Empirical studies have shown that *Erwinia* has a positive effect on thrips fitness, yet the transmission of these gut bacteria is likely not vertical and they are probably acquired from the plant surface (De Vries et al., 2004). *Pantoea* bacteria are also likely to be transmitted from mother to offspring via the plant and horizontally to other thrips through faeces, and persist in thrips through different life stages (Dutta et al., 2016). Based on this study, however, it is not known if thrips-associated bacteria confer any fitness advantage, so their condition as insect mutualists remains to be demonstrated. A more recent study on *T. tabaci* identified a very diverse gut bacterial community mainly consisting of Proteobacteria (Gawande et al., 2019), and in another the diversity and structure of the bacterial community was found to depend on the environment and habitat where thrips lived (Dickey et al., 2014). All these examples suggests that thrips symbionts are quite diverse and primarily acquired from their environment.

Preliminary sequencing on individual thrips performed at CBGP confirmed that French thrips are associated with diverse bacterial assemblages. In spring 2021 and 2022 we sampled thrips along seven different elevational gradients in France, from the Pyrenees

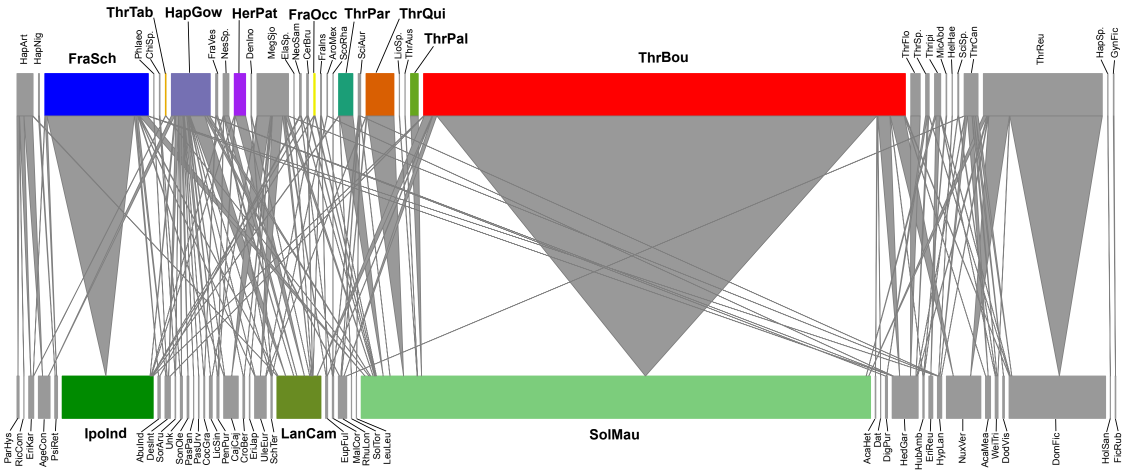


Figure 15. Quantitative bipartite flower-visiting network between plants and thrips obteined in the study by Dianzinga et al (2020). The length of the bars (plants at the bottom and insects on top) and the polygons connecting them are relative to the abundances of insects on plants. The most important species are shown in boldface and are abbreviated as follows: *Solanum mautirianum* (SolMau), *Ipomea indica* (IpoInd), *Lantana camara* (LanCam), *Frankliniella occidentalis* (FraOcc), *F. schultzei* (FraSch), *Haplothrips gowdeyi* (HapGow), *Hercinothrips pattersoni* (HerPat), *Thrips bourbonensis* (ThrBou), *T. palmi* (ThrPal), *T. parvispinus* (ThrPar), *T. quilicii* (ThrQui) and *T. tabaci* (ThrTab).

to the Alps stopping by Mont Aigoual and Mont Ventoux. Each transect ran from 0 to 15000 m.a.s.l, and at each transect we established six sites, each separated by 300m of elevation. At each site we sampled adult thrips from flowers on a total of nine different plant species. With this data and by sequencing bacteria from individual thrips, we will be able to build interaction networks of plant-thrips and of thrips-bacteria. We will test hypotheses on how these two different networks change with elevation. More in particular we hypothesise that networks will become dominated by generalist species (thrips on plants or bacteria in thrips) at higher elevations. This idea is partially supported by previous studies on insect-plant interactions that suggest that specialisation is a risky strategy at higher elevations. At higher elevation, resources are limited and therefore relying on a larger variety of energy sources may buffer species extinctions (Bommarco et al., 2010; Tylianakis & Morris, 2017). This idea has been tested (often with contradictory results) in networks of plant-pollinators (Classen et al., 2020; Librán-Embid et al., 2021), plant-herbivores (Morris et al., 2014; Pitteloud et al., 2020) and even in plant-microbes (Cobian et al., 2019), but as far as I am aware it has never been tested on insect-microbe interaction webs. This project will also provide novel insights into thrips symbiosis. We will, for example, assess whether thrips microbiomes vary depending on the food plant where insects were collected. These observations could point to specific bacteria as key players in host plant use and open the door to further studies where symbionts are manipulated, for example using antibiotics. Experimental approaches can be particularly useful with very polyphagous pest species like *F. occidentalis* to better understand the pest status of these species with regards to particular crops.

GENERAL CONCLUSIONS

As this document shows, I have developed a quite diverse research program. At the level of the model system I started with moths, then moved to aphids and finally to thrips. Not being loyal to a model system has its advantages, what we learn from one system can be novel in another and building bridges between models can allow proposing general principles. These advantages, however, come with costs because learning and managing novel models takes time, energy and lots of failures. Acknowledging failures in academia is important and it is a pity they are not often reflected in our curricula vitae, even if they are often unavoidable setbacks needed to advance. Even though I switched model systems many times, I have been quite loyal to the field of entomology through the lens of experimental community ecology. The field of community ecology is huge and I will focus in this final part of the manuscript on the applied perspectives of my research, which can be framed more precisely within the field of biocontrol. More general interests include the understanding of terrestrial ecosystems with the ultimate goal of anticipating and preventing the dramatic consequences that human impacts have on them. I will also highlight my future research perspectives and I will comment on my personal view on mentoring early career scientists. I will finally discuss my view on Open Access Publishing and on the biasses that man publishing in the Global North may have.

I have done most of my research studying biocontrol and insect community dynamics in population cages and in simplified field settings. As I have discussed before, these types of experiments have been key to advance our mechanistic understanding of species interactions. These approaches, however, come with some limitations that I will list in the following, together with potential solutions. (i) Dispersal and metacommunity structure are ignored and we know that what happens in a community is not independent of what happens in other communities connected via migration (Hanski & Gilpin, 1997). (ii) The way we set up communities is by including an initial set of organisms that we follow through time simply by adding new plants fully fertilised every week. We thus maintain the carrying capacity constant but ignoring that plant resources are depleted upon herbivore outbreaks with concomitant feedbacks on soil nutrients and mineralisation (e.g. Van der Putten et al., 2001, 2013). (iii) When we set up our experimental communities, we start with organisms with a limited amount of genetic diversity. We thus ignore (or substantially limit) eco-evolutionary dynamics in our experiments. I am aware of these limitations and some of my future research aims at overcoming them.

One of such approaches would be to perform experiments in ecotrons, which are advanced controlled environment facilities used to replicate experimental ecosystems (Roy et al., 2021). For example, dispersal could be emulated in the laboratory by connecting cages, but also in semi-field conditions like in the Metatron facility that the CNRS has built in Moulis (Legrand et al., 2012). In the Metatron several greenhouse-sized cages are available and they can be connected through corridors while manipulating temperature in each individual compartment. The CNRS has another ecotron, this one in Montpellier and in fact located a few hundred metres from my current office. In this ecotron soil conditions can be measured and manipulated together with environmental gases like Ozone or CO_2. Performing experiments in these facilities would be very exciting. Despite the advantages that ecotrons provide to ecological experiments, it would be unrealistic to expect that most ecological research is performed in these facilities. Ecotrons are expensive to build and to utilise. They are therefore not available to all scientists particularly for those in the Global South. As mentioned above, population cages or field plot experiments are also limited because the starting populations are usually quite poor in terms of genetic diversity; extremely poor if we work with aphid clonal lines. Specific experiments can be performed to test the eco-evolutionary potential of the studied communities for example by varying the amount of different genotypes in initial populations, as has already been done with aphids (Turcotte et al., 2011, 2013; Hafer-Hahmann & Vorburger, 2020) and other insects (Hufbauer et al., 2015). Another good alternative to perform experiments in more realistic scenarios is to scale-up experiments by moving from small population cages to larger ones or to real-world situations. This is exactly what we propose in the collaborative EnemyCocktail project that I just started as coordinator. We will engineer real trophic webs by releasing natural enemies in commercial greenhouses. The approach is as exciting as risky. Being able to engineer these webs seems quite a challenge. The enemies that we will release may not be abundant enough to have any relevant impact on local communities, or they may disperse. In addition, in this scenario local communities can evolve resistance to our released enemies, or bad weather may kill them before they settle.

One of the main applied aspects of my research has been to provide a better understanding of the complex ecological interactions that emerge when biocontrol techniques against pests are used. The potential advantages of expanding biocontrol techniques in agriculture are obvious. It is estimated that at least 30% of global agricultural production is lost due to arthropod pests (Oerke, 2006). Chemical insecticides are the single most important method to control these pests even if their use raises important concerns for public health, environmental pollution and the emergence of resistant pests (Lamichhane et al., 2017; Barratt et al., 2018; Bremmer, 2021). Biocontrol with natural enemies is a sustainable and lasting alternative to suppress pest populations, which has limited impact on fauna, beneficial organisms, consumers and stakeholders, while minimising the risk of emergence of resistant populations (Stenberg, 2017; Hajek & Eilenberg, 2018; Noriega et al., 2018). Biocontrol is therefore emerging as key for the success of the ecological transition in agricultural production (Hulot & Hiller, 2021). Important efforts are being made to speed-up and extend its implementation. Examples include national initiatives like the Ecophyto plan in France [10], which are reflected in the steady increase in the biocontrol industry during the last few decades (Lamichhane et al., 2017; Barratt et al., 2018; Hajek & Eilenberg, 2018; Bremmer, 2021; Hulot & Hiller, 2021; Riemens et al., 2021). Are these efforts enough? I have my doubts. The Ecophyto plan, for

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[10] https://agriculture.gouv.fr/le-plan-ecophyto-quest-ce-que-cest

instance, seemed to work with a 30% drop in the production of phytosanitary products between 2008 and 2021, but these numbers rebounded the year after [11]. I believe that plans like Ecophyto are interesting and useful but to reduce the use of insecticides a drastic shift in consumer habits is a must. To achieve this, a drop in the prices of those products originating from organic or integrated agriculture is necessary and I hope that my research in the ecology of plant-insect-enemy interactions will contribute to reaching this goal.

If the situation in agriculture does not progress as quickly as we would like, the situation in natural ecosystems does not look much better. We live in an era where all ecosystems are facing unprecedented changes associated with human activities. As a consequence one out of six species is likely to be extinct by the end of the century. Many recent studies report marked declines in insect abundance and diversity across ecosystems and taxa (Hallmann et al., 2017; Leather, 2018; Habel et al., 2019; Montgomery et al., 2020) and there is currently clear evidence that insects are declining and have done so for years in many habitats around the world. These declines are driven by changes in land-use, pollution, biological interactions (mostly invasions), and climate change (Seibold et al., 2019; Sánchez-Bayo & Wyckhuys, 2019; Cardoso et al., 2020; Wagner, 2020; Wilson & Fox, 2020). Ecologists play an important role in understanding the mechanisms behind these losses, to anticipate future extinctions and to propose mitigating strategies (Hooper et al., 2012; Yvon-Durocher et al., 2015). A community-oriented approach is fundamental to achieve such a purpose. Human impacts on species are usually transmitted through interactions with other species in the trophic web, and the dynamics of any species can only be understood if the community the species is embedded within is taken into consideration. This understanding can not ignore the many complex interactions I discussed in this document. For example, some authors estimate that up to 80% of the species extinctions are indirect (i.e. they are triggered by the loss of other species) or due to indirect effects, a phenomenon known as extinction cascades (Saterberg et al., 2013; Brodie et al., 2014). As Daniel H. Janzen once said ‘‘What escapes the eye, however, is a much more insidious kind of extinction: the extinction of ecological interactions'' (Janzen, 1974). With my long-term collaborator Dirk Sanders we have recently written a couple of review papers (one under review) on these topics. In the first one we discuss how indirect effects may underlie insect declines (Kehoe et al., 2020), in the second we provide examples of how these same effects may determine the way organisms engineer ecosystems and change their properties [12]. These papers show my increasing interest in studying the role that complex interactions play in maintaining biodiversity but also in triggering species declines. As with the use of ecological pest control methods, the strategies that our society is taking to mitigate the degradation of most ecosystems on Earth seem insufficient to prevent the catastrophic situation that is approaching. Desperate and legitimate militant

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11-https://www.lemonde.fr/en/france/article/2023/03/01/french-pm-sets-out-to-coordinate-pesticide-measures-with-the-eu_6017775_7.html

12 Dirk Sanders and Enric Frago. Ecosystem engineers in ecological networks: implications for community stability. Invited review at Functional Ecology (under review).

initiatives like *Extinction Rebellion*[13] or *Les soulèvements de la terre*[14] are raising awareness particularly in young generations. As an ecologist and as a dad I believe I have the obligation to act soon, but I fear our generation is too spoiled to act with the strong commitment the situation requires.

All the work I described above would have not been possible without many other scientists and biocontrol practitioners with whom I have worked with. I have collaborated and supervised many early career scientists including MSc, BSc and PhD candidates. I have tried my best to be a good mentor for them by providing a smooth transition from where the person came to where the person wanted to go. For each early career scientist this path was different, from purely academic scientists that wanted to pursue a career in academia, to those that wanted to settle in the private sector, including many that were not sure about what they wanted to do. I have been in many laboratories and at every step I have learned a lot of what a good mentor can provide but also what a good supervisor should not do. Putting purely the interests of the main researcher (or of a whole research project) before that of the early career scientist is probably the most important conflict to avoid but likely the most difficult not to get caught up in. In this regard I have recently pledged to the #MentorFirst initiative initiated by Jennifer M. Heemstra and Neil K. Garg [15]. The initiative states that "*As science faculty, we are expected to produce high-quality scientific research. While this is a central goal, it is accomplished by leading teams of students, postdocs, and research staff. Thus, our success in producing research is inextricably linked to the significant role we play as mentors to the researchers in our labs. We believe that prioritizing the needs of researchers and focusing on providing high-quality mentorship leads to greater creativity, and ultimately productivity, in our research programs*". By taking the #MentorFirst Pledge, I made "*a commitment to prioritize the professional development, career goals, and general well-being of the members of your research lab as they develop as scientists and pursue their personal and professional goals*".

An important part of our research implies making our results available to the scientific community. To do so, we publish in peer-reviewed journals. The last few years have seen the beginning of what could become a paradigm switch in academic publishing. Open Access publishing and the public access to data is increasingly requested by funding agencies and public institutions like the European Union following FAIR principles of data Findability, Accessibility, Interoperability, and Reusability. Many voices are also shouting against the commodification of scientists' work by for-profit Editorials. One of such examples is DAFNEE, a Database of Academia Friendly jourNals in Ecology and Evolution [16], which lists 398 non-profit, learned society or university-associated journals relevant to the field of ecology and evolutionary biology. Some even more appealing strategies are also growing. I am particularly excited about the *Peer Community In* (PCI)

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[13] https://rebellion.global/

[14] https://lessoulevementsdelaterre.org/

[15] https://mentorfirst.org/

[16] https://dafnee.isem-evolution.fr/

initiative [17], a "*free recommendation process of scientific preprints based on peer reviews and a journal*". I have already collaborated with this initiative and I will keep doing so in the future. In this novel system preprints are sent and evaluated by peers and then recommended. After recommendation, articles can be sent directly to traditional journals or to the Peer Community Journal, which is free to read and to publish. This latter point is crucial to me because many Open Access journals impose prices that are not affordable for most scientists particularly in the Global South. To boost this initiative, PCI launched the PCI manifesto that states that "*I agree to submit at least one of my best articles to a PCI for peer review before the end of 2023 and, if recommended, to publish it in Peer Community Journal.*"; "*I support PCI and adhere to the idea of making Peer Community Journal a widely-used venue for the publication of high-quality articles.*"; "*I will be bound by this promise only if at least 500 other researchers make the same commitment.*" So far the manifesto has been signed by more than 1000 researchers, me included.

To finish I would like to give a gender dimension to my research. Since the raise in of the #MeToo movement in 2017 the expectation that our society embraces safety and fights discrimination openly has become a reality. Societies for the study of ecology and evolution have not been an exception. Having a LGBT mixer in conferences, for example, is becoming the norm rather than the exception. These initiatives had a strong impact on the way I currently conceive research. I did my PhD under the supervision of three men and the two principal investigators of my postdocs were men too. The first six papers I published were authored exclusively by men. Did this happen by chance? I don't think so. We are all extremely biassed and realising it may help us to take a step back and overcome these biases. My list of co-authors is still quite biassed towards men but increasingly more women are included both in my manuscripts and my projects. There are many ways parity can be reached, and I am increasingly putting in practice such strategies. One easy way of reaching parity in research groups or projects is by considering our biases when recruiting early career scientists by setting-up mixed selection committees. This is a common procedure at every recruitment I lead.

Since the beginning of my PhD I have seen a dramatic change in many aspects in academia and in insect research. Open Access publishing is becoming a reality, we are more aware of discrimination and many experiences show that growing crops without pesticides is possible. We are still a long way to fully reach these goals and I hope that my research program may contribute to this end. This program involves a solid research program on experimental ecology that goes hand in hand with a solid investment in ensuring a caring research environment for early career scientists and for the generations to come.

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[17] https://peercommunityin.org/

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PART III: TIRÉS À PART

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RESEARCH PAPER

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The interacting effect of habitat amount, habitat diversity and fragmentation on insect diversity along elevational gradients

Niry T. Dianzinga[1,2] | **Marie-Ludders Moutoussamy**[1] | **Joëlle Sadeyen**[2] | **Lala H. R. Ravaomanarivo**[3] | **Enric Frago**[1,4,5]

[1]CIRAD-UMR PVBMT, Saint-Pierre, La Réunion, France

[2]Université de la Réunion-UMR PVBMT, Saint-Denis, La Réunion, France

[3]Département d'Entomologie, Faculté des Sciences, Université d'Antananarivo, Antananarivo, Madagascar

[4]CIRAD, CBGP, Montpellier, France

[5]CBGP, CIRAD, INRA, IRD, Montpellier SupAgro, University Montpellier, Montpellier, France

Correspondence
Enric Frago, CIRAD/UMR CBGP, 755 avenue du campus Agropolis - CS30016 34988 Montferrier sur lez cedex, France
Email: enric.frago@cirad.fr

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Abstract

Aim: Elevational gradients are a useful approach to evaluate how environmental factors affect animal diversity. Decades of studies on the elevation-diversity gradient have revealed that this gradient varies greatly with taxa and geographic regions. One potential explanation for this may be the dependence of the relationship on landscape features. We explore the impact of fragmentation, habitat diversity and habitat amount on insect diversity (alpha and beta) and abundance along elevational gradients. We hypothesize that insect diversity and abundance will relate negatively with elevation, but positively with these landscape features. We also hypothesize that landscape features will interact in a way that the positive effect of a given variable on insect diversity may be offset by the others.

Location: Reunion Island (Indian Ocean).

Taxon: The insect order thrips (Thysanoptera).

Methods: Insects were sampled along replicated elevational gradients, and at each sampling plot landscape features and abiotic variables were estimated within buffers surrounding the site. Insect alpha diversity was estimated using abundance-based rarefaction methods, whereas beta diversity was estimated calculating the "Local contributions to beta diversity" metric. The effect of elevation, rainfall, landscape features and their interactions was assessed on insect alpha and beta diversity and abundance during two consecutive seasons using linear mixed effects models.

Results: We found that thrips alpha and beta diversity was negatively related with elevation, but the relationship varied between seasons and rainfall regimes. Among the different landscape features considered, we found that habitat amount had the strongest effect on diversity. The effect of habitat amount on diversity, however, was offset in areas of low habitat (or land cover) diversity.

Main conclusions: Generalizing the factors that underlie the elevation diversity gradient has become a cornerstone in ecological theory because it can help to understand the impact of human activities on diversity. Here we show that taking landscape information into account may help to fulfil this objective because landscape effects co-vary with elevation with often intricate consequences for diversity.

KEYWORDS
alpha diversity, beta diversity, elevational gradient, environmental gradient, multi-scale analysis, barcoding, seasonality, insect, thrips, Thysanoptera

1 | INTRODUCTION

It is nowadays widely accepted that human activities are triggering unprecedented biodiversity losses in most terrestrial ecosystems. Human activities usually have a negative impact on biodiversity by directly degrading and transforming natural habitats, but also indirectly through changes in global climatic conditions (Barnosky et al., 2011; Cardinale et al., 2012; Hooper et al., 2012; Urban, 2015). By comparing diversity across space and assessing its changes in relationship with climate, relief and landscape configuration ecologists seek to understand and predict large-scale impacts of human activities on biodiversity. To achieve this, elevational gradients are useful because assemblages of species found at very different conditions can be studied within small geographic distances (Hodkinson, 2005; Sundqvist, Sanders, & Wardle, 2013; Tylianakis & Morris, 2017). Several environmental factors vary with elevation leading to lower plant species richness and structural complexity at higher elevations (Kreft & Jetz, 2007). This elevation-diversity gradient is observed in insects because they are affected by environmental factors too, but also because they tightly depend on plants. This gradient, however, often varies with climatic regions and insect taxa (Chamberlain, Brambilla, Caprio, Pedrini, & Rolando, 2016; McCain, 2009; Sundqvist et al., 2013). Most studies have explored how species richness and evenness (i.e. alpha diversity) change with elevation, and there is an increasing interest in how species composition (i.e. beta diversity) changes along with these gradients. While alpha diversity expresses net diversity differences among species assemblages, through the lens of beta diversity, differences in the composition of insect assemblages can be assessed, and areas where unique species are found may be pinpointed (Mori, Isbell, & Seidl, 2018; Socolar, Gilroy, Kunin, & Edwards, 2016).

The elevational-diversity gradient was already noticed by Alexander von Humboldt in the 18th century, and since then it has been intensively studied with mixed and often contradictory results. Generalizing the factors underlying this gradient across taxa and geographic regions has become a cornerstone in ecological theory, particularly because it can help to assess and to forecast the impact of human activities on diversity (Hodkinson, 2005; Sundqvist et al., 2013; Tylianakis & Morris, 2017). The elevational-diversity gradient varies greatly with taxonomic groups and climatic regions because the impact of elevation on diversity depends on complex interactions among environmental variables and habitat and landscape characteristics (Chamberlain et al., 2016; McCain, 2009; Sundqvist et al., 2013). This variation also depends on human activities, which are usually greater at lower elevations where human populations concentrate (Luck, 2007). Landscape features have known impacts on insect diversity and are thus important to understand the elevation-diversity gradient. The most important landscape features with known impacts on animal diversity are habitat diversity, habitat amount and fragmentation. As revealed in a meta-analysis by Stein, Gerstner, and Kreft (2014), diverse or heterogeneous habitats often contain a larger number of species because they provide a larger range of abiotic conditions, available niches and refuges. These conditions ultimately allow species coexistence, reduce extinctions and promote speciation. Habitat diversity has been strongly reduced by human activities particularly due to the homogenizing effect of extensive cropping, forestry and livestock production (Kremen & Merenlender, 2018). Despite the results found in the meta-analysis, evidence of negative or neutral effects of habitat diversity on species diversity also exist (Bertrand, Burel, & Baudry, 2016; Marini, Prosser, Klimek, & Marrs, 2008). The effect of habitat amount on diversity, on the other hand, is clearly positive. When the amount of habitat that species can exploit is reduced, species struggle to persist because resources are of less quality and less abundant, and because critical resources may be lacking (Samways, McGeoch, & New, 2010). Habitat loss is one of the landscape features most strongly influenced by human activities, and is considered as one of the main drivers of current biodiversity loss (Cardinale et al., 2012; Mantyka-pringle, Martin, & Rhodes, 2012). Fragmentation too affects species diversity, although whether the effect is positive or negative remains a controversial topic. Human activities are important drivers of fragmentation. Fragmented landscapes are often composed of small patches of natural habitat surrounded by urban, agricultural or timber-producing lands. Most authors consider that fragmentation has a negative effect on species diversity (Fletcher et al., 2018) because it increases the division and isolation of natural habitats thus exposing them to disturbing human land uses (Haddad et al., 2015). Fahrig (2017, 2019), however, suggests that since habitat fragmentation often comes together with habitat loss, when the amount of habitat available for organisms to exploit is taken into account (an estimate known as fragmentation *per se* [sensu Haila and Hanski (1984)]), the negative effect of fragmentation is lost. This idea was proposed as the habitat amount hypothesis (Fahrig, 2013) [but see criticism by Fletcher et al. (2018) and Hanski (2015)], where she suggests that habitat amount and fragmentation can be measured independently so that their relative contribution to diversity can be estimated. A meta-analysis that encompasses 35 studies from different locations around the globe has recently provided support for this hypothesis (Watling et al., 2020).

Human impacts on natural habitats vary in intensity with elevation, but their effect on different landscape features is often intricate. For instance, while most human populations concentrate at lower elevations (Luck, 2007), food, timber and livestock production usually concentrates at particular elevational ranges, with concomitant effects on habitat diversity and fragmentation. Even if diversity usually decreases with elevation, speciation and endemism may be larger at high elevations where habitats are more isolated and, in temperate areas, more fragmented due to glacial advances and retreats (Steinbauer et al., 2016; Vetaas & Grytnes, 2002). In addition, landscape features are usually interconnected. The positive effect of fragmentation on species diversity, for instance, may be linked to habitat diversity because fragmented landscapes are usually more heterogeneous and structurally more complex (Fahrig, 2017). A recent theoretical model showed that habitat amount modulated fragmentation effects on diversity so that fragmentation effects were positive when the amount of habitat was large, but negative when habitat amount was reduced (Rybicki, Abrego, & Ovaskainen, 2020). In another example, Corcos et al. (2018) found that the diversity of four different insect guilds was affected both by elevation and habitat diversity, but the effect of one variable never modulated the effect of the other. Despite all these recent reports, few studies have so far measured the relative contribution of different landscape attributes to diversity (Fahrig, 2019; Hanski, 2015), and how they interact with elevation. This knowledge is important to evaluate whether diversity changes along elevation are mostly driven by abiotic conditions or the local landscape. Exploring interacting effects among landscape attributes is particularly important to better understand whether the impact of habitat loss may be exacerbated or buffered by changes in habitat diversity or fragmentation. This knowledge may ultimately help better transferring biogeography theory into conservation and management practice.

In this study we explore the relative contribution of elevation, environmental variables and landscape features to Thysanoptera (i.e. thrips) diversity. Thrips are slender insects, of approximately 1 to 3 mm long with about 6.000 extant species described worldwide (Stork, 2018). Thrips larvae can be herbivorous, predatory or fungivorous, and many species disperse to flowers as adults to feed on pollen or to mate (Mound, 2005). This study was performed in Reunion, a small volcanic island located in the south-western Indian Ocean. Local-landscape characteristics were obtained by estimating habitat diversity, habitat amount and fragmentation using highly-resolved vector layers and establishing buffers around sampling points. Habitat diversity was assessed as land cover diversity (Stein et al., 2014), fragmentation as the length of the perimeter of the different polygons found within the buffer, and habitat amount as the proportion of potentially suitable habitat within the buffer (Watling et al., 2020). Since insects were sampled from flowers and during two consecutive seasons, the role of the plant, season and rainfall were also taken into consideration. The following hypotheses are tested (Figure 1): (a) We hypothesize that alpha diversity (i.e. species richness and evenness), insect abundance and beta diversity will decrease with elevation. (b) We hypothesize that habitat amount and habitat diversity will relate positively with insect diversity and abundance. Since the proportion of suitable habitat is taken into account in our study, our estimate of fragmentation can be considered as fragmentation *per se*, and we thus hypothesize that this feature too will relate positively with insect diversity and abundance. (c) We also explore interactions among the different landscape features, and in particular whether the relationship between habitat amount and variables related to insect diversity and abundance is modulated by either habitat diversity or fragmentation. We hypothesize that the positive effect of habitat amount on insect diversity may be offset in low diversity habitats Fragmentation, on the other hand, may have an either positive or negative effect on the relationship between habitat amount and species diversity. The interaction between habitat diversity and fragmentation may provide a better understanding

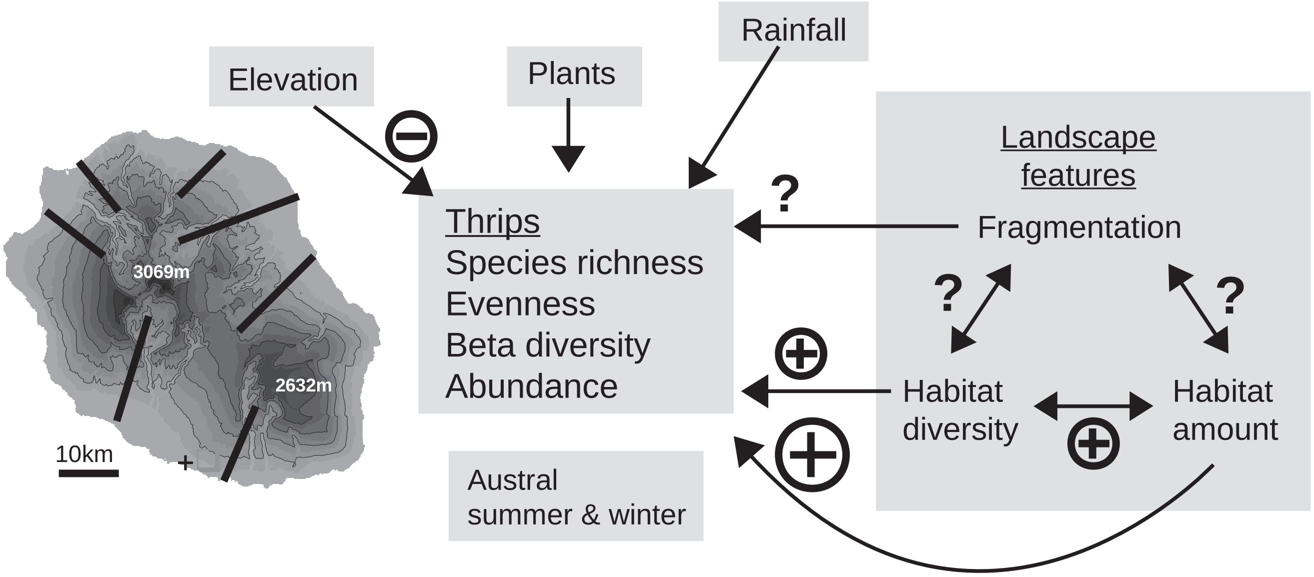


FIGURE 1 Conceptual diagram of the experimental design and variables tested in this study. Arrows point from predictor to response variables, whereas double-headed arrows indicate interactions between landscape variables. Positive and negative symbols represent our a *priori* hypotheses on the effect of elevation,landscape features, and their interactions on insect diversity and abundance. Larger positive symbols represent expected stronger effects on diversity and abundance, whereas question marks are shown when no directional hypothesis is proposed. A map of Reunion island with 500m contour lines and the location of transects is also shown.

of whether fragmentation effects operate via changes in habitat diversity.

2 | MATERIALS AND METHODS

2.1 | Study system

In this study, adult insects belonging to the order Thysanoptera (i.e. thrips) were sampled from flowers along replicated elevational gradients that run across different habitat types. Thrips are small insects with about 6.000 extant species (Stork, 2018), and were sampled from flowers because even if larvae can be herbivorous, predatory or fungivorous, many species feed on pollen or disperse to flowers for mating (Mound, 2005). This study was performed in Reunion, which is located at 55°39′ E, 21°00′ S in the south-west of the Indian Ocean between Madagascar and Mauritius with 2,512 km^2 of surface. The island has a maximum diameter of 70 km. Its rugged terrain reaches 3,070 m.a.s.l. and 2,631 m.a.s.l. in the Piton des Neiges and Piton de la Fournaise, respectively. The island has a tropical climate with two seasons: summer and winter. The former occurs from November to April and it is rainy and hot, while the latter occurs from June to September and it is less hot and dryer. Climatic conditions are strongly dependent on the topography and trade winds (i.e. regular ocean winds blowing from East to West). Mean annual rainfall is higher in the eastern part (windward coast) with 1,500 mm to > 8,000 mm, whereas in the western part (leeward coast) annual rainfall varies between <500 mm to <1,500 mm. Mean annual temperatures are of 24°C near the coastline, and they can descend to <12°C at 2,000 m.a.s.l. This island is considered a global hot-spot of diversity (IUCN, 2008) and is typified by a high level of endemism, with 46% of its 1.712 species of vascular plants endemic and with eight endemic genera. Among arthropods, it is estimated that 40% of beetles and 25% of spiders are also endemic (IUCN, 2008). Urbanization and agricultural activities are mostly concentrated below 1,000 m, while the largest proportion of natural habitats (c. 30%) is mostly concentrated above 1,200 m in areas that are less inhabited and often protected under the umbrella of the National Park (Strasberg et al., 2005). As in most tropical islands (Harter et al., 2015; Russell & Kueffer, 2019), the main threats to this biodiversity are global changes, urbanization, agriculture and alien plant invasions, the latter three being particularly important at low elevations (Strasberg et al., 2005).

2.2 | Sampling design and insect collection

Thrips were collected along seven replicated elevational transects (Figure 1). Transects were selected to capture landscape diversity and the different vegetation zones, and to ensure that landscape features varied with elevation but not collinearly. Reunion is a small island, and to avoid higher elevation points to converge, sampling took place below a mid elevation point of 1,600 m. Three transects reached up to 1,400 m, and four to 1,600 m. Sampling sites were established every 200 m of elevation, these elevational ranges being approximate as sites were selected based on visual detection of abundant plants at the flowering stage. At each site, insects were sampled from all flowering plants in a circular area of 30 m of diameter using the beating sheet technique with a rectangular 40 × 30 cm white plastic tray and a mouth aspirator. To standardize sampling effort per plant, each was sampled for 10 min. Usually a single site per season, elevational range and transect was selected, but sometimes up to three sites per elevational range were established when few plants in bloom were found. Thrips were sampled in 2017 in summer (January to February, 64 sites) and winter (May to July, 56 sites) (Appendix S1) on sunny, dry and non-windy days from 9 a.m. to 3 p.m. Since most plants only bloom in one of the two seasons, sampling sites were not the same in winter and summer and the two seasons were therefore analysed independently. Sampling was carried out by N.T.D., M.-L.M. and E.F. To avoid any sampling bias, at each site sampling of the different plant species was randomly assigned to one of the three researchers. At each site, latitudinal and longitudinal coordinates were recorded, and elevation was obtained with the R function *elevation* from the package *rgbif* version 1.0.2.

In this study the unit of analysis was the assemblage of insects obtained from a given plant. Site was not the unit of analysis because the number of plants (and hence sampling effort) varied between sites. Plant flowers were thus microhabitats from where adults were obtained because even if adults of most species disperse to flowers to feed on pollen, or to mate, their larvae feed on other plant species, fungi, or on other arthropods (Mound, 2005). Taking the habitat amount hypothesis as an example (Fahrig, 2013), diversity patterns should be studied by exploring "the number of species in a plot of fixed size" and how they vary with "total habitat area in the ‘local landscape’ surrounding the plot”. In our study, flowers represent the plots of fixed size, whereas the information extracted from buffers (see below) represents the local landscape.

2.3 | Morphological and molecular insect identification

A short description of the methods used to identify insects is provided here, but more details can be found at Appendix S2. Insects were first identified morphologically by N.T.D and a subset of specimens barcoded to ensure identifications and to detect potential cryptic species. Based on their abundance and the potential presence of cryptic species [as in *Frankliniella schultzei* (Tyagi et al., 2017)], a total of 223 specimens representing four known endemic species [*Thrips bourbonensis*, *T. candidus*, *T. quilicii* and *T. reunionensis* n. sp (Goldarazena, Dianzinga, Frago, Michel, & Reynaud, 2020)] and six species with a global distribution (*T. parvispinus*, *T. florum*, *Megalurothrips sjostedti*, *F. schultzei*, *Hercinothrips pattersoni* and *H. gowdeyi*) were barcoded. 196 sequences were obtained and new ones imported from NCBI-GenBank (https://www.ncbi.nlm.nih.gov/genbank/) to build a phylogenetic tree using the maximum likelihood method. All specimens, sequenced voucher specimens and their DNA are currently stored at the entomological collections of CIRAD-UMR PVBMT (CIRAD, Saint-Pierre, La Réunion) and at Centre for Biology and Management of Populations (CBGP-INRA, Montpellier/Montferrier-sur-Lez, France).

2.4 | Estimation of landscape features and environmental variables

Habitat diversity, habitat amount and fragmentation were obtained from five nested circular buffers surrounding sampling sites using a highly-resolved vector layer of Reunion Island land cover map (http://aware.cirad.fr/layers/geonode%3Aclassif_gabir_2016_2017). Vector-layer information was extracted using *QGIS* Desktop version 2.18.2 and then exported to R. The vector layer used contained nine habitat types: sugar cane, vegetable crops, forest, forest plantation, grassland, bare rock, savannah, orchards and urban habitats. Habitat diversity was estimated with the Shannon index as the proportional contribution of the nine habitat types within the buffer [i.e. land cover diversity, Stein et al. (2014)] using the function *diversity* from the package *vegan*]. Fragmentation was estimated as the length of the perimeter of the different polygons found within the buffer divided by the total surface of the buffer. Habitat amount was estimated as the proportion of the total surface of the buffer that was considered as habitat, a method recently proposed by Watling et al. (2020). Even if the information provided by land cover data can be complemented with additional site data (Betts et al., 2014) like plant diversity, land cover maps are ideal to capture the habitats potentially used by functionally-diverse animal groups. This is particularly true in this study given the different ecosystems studied, the abrupt landscape of the island, and the variable feeding habits of the insects studied (as explained above). Landscape features considered as non-habitats were urban areas, bare rock or the ocean (this latter case was common in sites located near the coast). Urban zones were not considered as thrips habitats because although some thrips species can be found in ornamental trees, the biomass of vegetation in these areas is usually low. If habitat amount is estimated considering urban zones as habitat, some minor differences are found, but the main results remain similar (results not shown). Environmental variables were obtained for each sampling site and season using the raster layers of average monthly data from 2007 to 2017 in Reunion Island (Météo-France, https://donneespubliques.meteofrance.fr/). The data obtained was mean, maximum and minimum annual temperature, and accumulated annual rainfall, which was extracted from raster layers using the function *raster* from the package *raster* version 2.7–15.

2.5 | Statistical analyses and multi-scale analysis

We performed all statistical analysis with *R* software version 3.4.2 using several packages (see R package details in Appendix S3). Alpha diversity was estimated using abundance-based rarefaction methods to estimate Hill numbers (or effective number of species) and to take into account potential undetected species of thrips (Chao et al., 2014; Chao & Jost, 2012; Colwell et al., 2012; Jost, 2006). Species richness and the Simpson index (i.e. evenness) were estimated as Hill numbers of order $q = 0$ and $q = 2$, respectively, using the *iNEXT* function from the package *iNEXT*. The package *adespatial* was used to assess the ecological uniqueness of each insect assemblage based on their contributions to global beta diversity, by calculating the 'local contributions to beta diversity' (LCBD) index (Legendre and De Cáceres, 2013). To test the effect of elevation, landscape features, and environmental variables on diversity and abundance estimates, linear mixed effects models were built with a Gaussian error distribution using the function *lmer* from the package *lme4*. As explained above, in our study the unit of replication was the thrips obtained in a given plant. We assumed a linear relationship among variables as non-linear relationships were not observed (Appendix S4). An independent model was built for each of the following response variables: species richness (i.e. number of species), the Simpson index (or species evenness), beta diversity (the LCBD index), and insect total abundance. Given the large variation in environmental conditions during winter and summer, and that different sites were established during these two seasons, independent models were also built for the two different seasons studied. In each model plant identity, elevation, precipitation, fragmentation, habitat diversity, habitat amount, and pairwise interactions between landscape features were included as explanatory variables (Figure 1). As environmental variables, precipitation, minimum, mean and maximum temperatures were obtained, but only precipitation was retained in the models as the other variables were highly correlated with elevation (Pearson correlation $R \geq 0.95$, $p < 0.001$, Appendix S5). Our study does not particularly focus on plant effects on thrips diversity and abundance, but this variable was included in all models as co-variable to account for this source of variability. Plants that were sampled less than five times during the whole study were considered as a single category (see details on sampled plants in Appendix S6). To account for the spatial autocorrelation between study sites, and for the non-independence of plants sampled in the same site, site nested within transect was included as a random effect. To further correct for any potential spatial autocorrelation a grid of cells of 0.09 × 0.09 decimal degrees was defined over the map. These cells were given a random identity, which was then included as a second random factor to ensure that the potential resemblance between nearby sites is accounted for. To improve model fit and interpretation, all continuous explanatory variables were standardized to mean and standard deviation equal to zero and one, respectively (Harrison et al., 2018). In the models for insect abundance, richness and Simpson diversity, the response variable was log transformed (expressed as log[response + 0.5]) to satisfy model assumptions. In addition, the *lmerControl* function with the optmizer *nloptwrap* from the package *nloptr* was used to improve model performance (Bates, Mächler, Bolker, & Walker, 2015). Model fit was assessed by visual inspection of the residuals. In the models for insect richness and evenness, model residuals revealed slight evidence of non-homogeneity, which was considered as having negligible effects on our model estimates as recently suggested by Schielzeth et al. (2020). The absence of data points with high influence was checked by calculating Cook's distances with the function *CookD* from the *predictmeans* package. Our models did not have any significant spatial autocorrelation among data points as revealed by the function *Moran-I* from the *ape* package. *p*-values were obtained with a Chi-square test, using the function ANOVA from the package *car*. Significant results were plotted using the function *visreg* from the *visreg* package, which allows plotting partial residuals plots from mixed effects models. The non-linear multidimensional scaling (NMDS) was used to visualize how thrips diversity is influenced by elevation, rainfall and landscape features, and also to visualize how specific insect species associate with specific plants or with particular environmental or landscape features. This analysis was performed by transforming data with the Hellinger transformation (Legendre and Anderson, 1999; Legendre and Gallagher, 2001) and using the *capscale* function from the package *vegan* version 2.5-1 . Since we had little information on dispersal capabilities of the studied insects, and thus of the scale at which they may be affected by landscape features (i.e. the scale effect), a multi-scale analysis was also performed. As suggested by Fahrig (2013), Jackson and Fahrig (2015) and Watling et al. (2020) this analysis was done by estimating the effect of landscape features using five nested circular buffers with five different radii from the centre of each sampling site: 100, 300, 600, 1,000 and 3,000 m. These distances to the centre of the sampling site may represent short and long-distance dispersal of thrips. It is expected that the variance explained by the different landscape features in statistical models is highest for the scale that best fits the home ranges or life dispersal of the species studied. In this analysis, the variance explained by the models was obtained with the function *r.squaredGLMM* from the package *MuMIn*, and significance of a given landscape feature in the different models was tested by comparing simplified and complex models (see more details in the Appendix S12). For the models using the same response and predictor variables *p*-values were corrected for multiple testing using the false discovery rate method. This method is less stringent than other methods like Bonferroni, and is appropriate when a large number of comparisons are performed (Benjamini & Hochberg, 1995).

3 | RESULTS

3.1 | Morphological and molecular identification of thrips

In this study, 4,280 specimens were collected and 4,261 identified to species level from flowers of 106 different plants: 86 exotic and 20 indigenous belonging to 44 families (Appendix S6). Insects were classified into 40 species, and we were unable to identify to species level 64 individuals (1.5%), which belonged to 19 different morphospecies. Seventeen of these morphospecies were identified to genus level (Appendix S7). The three known endemic species were well-represented with 1964 *Thrips bourbonensis*,150 *T. quilicii* and 114 *T. candidus*. A total of 329 specimens belonged to a species newly discovered in this study. This species has been named *T. reunionensis* n. sp (Goldarazena et al., 2020), and is very similar morphologically and at the molecular level to *T. bourbonensis*. Some widespread (and probably exotic) species were also very abundant, including *Frankliniella schultzei* with 518 individuals, *Haplothrips gowdeyi* with 381 individuals, *Megalurothrips sjostedti* with 199 individuals, and *Hercinothrips pattersoni* with 198 individuals. Association between plants and particular thrips species can be visualized in the non-linear multidimensional scaling analysis (NMDS, Appendix S8). For example, the newly discovered *T. reunionensis* (Goldarazena et al., 2020) was associated with the endemic plant *Dombeya ficulnea*, the endemic *T. bourbonensis* was mostly found at higher elevations, and the exotic and widespread *F. schultzei* was mostly found on the exotic plants *Lantana camara*, *Solanum mauritianum* and *Ipomea indica*. Maximum-likelihood trees (Appendix S9) mostly confirmed our morphological identifications. The endemic species *T. bourbonensis*, *T. candidus*, *T. quilicii* and *T. reunionensis* formed different clades, which were supported by 88, 100, 100 and 100 Ml bootstrap, respectively. However, two different evolutionary lineages were found for *F. schultzei*. Based on previous studies these two lineages were considered as different species and classified based on their colour (Tyagi et al., 2017). These lineages are known as *F. schultzei* Ia1, which is close to *F. schultzei* from India, and *F. schultzei* IIa1, which is close to *F.* cf. *schultzei/sulphurea* from Australia.

3.2 | Landscape changes along elevation and multi-scale analysis

Transects run from 0 to 1,600 m.a.s.l., and along this gradient important landscape changes were reported (Appendix S10 and S11). The mean proportion of natural forest increased steadily with elevation from mean values of 25% at low elevations to more than 75% above 1,200 m.a.s.l. The proportion of urban areas followed the opposite pattern as low elevation sites were surrounded by landscapes containing between 40% and 50% of urban landscape, but this proportion decreased gradually to less than 10% at higher elevations. Although the mean proportion of sugar cane and of meadows never represented more than 25% of the area surrounding study sites, these two habitat types were important between 500 to 1,200 m.a.s.l. Savannas are common in the west of the island, and represented an average of around 25% at 200 m.a.s.l. The mean proportion of area occupied by orchards was more important at low elevations, but they never represented more than 10% of the area. Other habitat types including forest plantations, vegetable crops or bare rock never occupied more than 5% of the area surrounding study sites (Appendix S10).

The different landscape features measured also changed with elevation without any remarkable difference when estimated using 300 and 1,000 m buffers (Appendix S11). Fragmentation changed with elevation but following a hump pattern that peaked

TABLE 1 Effect of elevation, plant of collection, accumulated rainfall, habitat diversity, fragmentation, habitat amount and pairwise interactions between habitat features on thrips alpha diversity (species richness and evenness), beta diversity (local contribution to beta diversity, LCBD) and abundance when extracting landscape data from buffers of 300m and 1000m of radius. A different linear mixed effects model was built for each buffer, and diversity and abundance estimates in both summer and winter. Significant P-values are presented in boldface type. Significant effects are graphically represented in Figure 4 as indicated near significant P-values. The total amount of variance explained by each model is also shown. Results using buffers of 100m, 600m and3000m of radius are shown in Appendix S13.

	Richness				Evenness				LCBD				Abundance		
	Chisq	*df*	*p*-val		Chisq	*df*	*p*-val		Chisq	*df*	*p*-val		Chisq	*df*	*p*-val
300 m BUFFER															
Summer															
Host plant	11.36	6	.078		10.98	6	.089		**41.59**	**6**	**<.001**		**28.02**	**6**	**<.001**
Elevation	0.13	1	.714		0.17	1	.684		**12.31**	**1**	**<.001**	**4B**	0.37	1	.543
Precipitation	**5.97**	**1**	**.015**	**4C**	**4.95**	**1**	**.026**		1.30	1	.255		0.08	1	.778
Habitat diversity	1.97	1	.161		0.42	1	.515		0.04	1	.837		0.83	1	.363
Fragmentation	1.33	1	.249		0.71	1	.401		0.34	1	.560		2.58	1	.108
Habitat amount	0.03	1	.852		1.36	1	.243		0.09	1	.767		0.19	1	.659
Hab. div × Fragm.	0.39	1	.532		0.28	1	.597		0.05	1	.821		0.25	1	.620
Hab. div × Hab. am.	**5.65**	**1**	**.017**	**4E**	**6.20**	**1**	**.013**	**4F**	0.03	1	.866		3.28	1	.070
Fragm. × Hab. am.	0.04	1	.843		0.01	1	.998		<0.01	1	.972		0.18	1	.673
Total variance explained			.331				.264				.405				.222
Winter															
Host plant	6.17	7	.520		**22.60**	**7**	**.002**		**94.23**	**7**	**<.001**		**80.40**	**7**	**<.001**
Elevation	3.13	1	.077		**7.79**	**1**	**.005**	**4A**	2.28	1	.131		0.67	1	.413
Precipitation	0.55	1	.460		1.09	1	.296		0.21	1	.648		0.05	1	.818
Habitat diversity	0.41	1	.523		0.36	1	.546		1.64	1	.200		0.01	1	.910
Fragmentation	0.13	1	.721		0.04	1	.832		3.43	1	.064		0.58	1	.445
Habitat amount	0.18	1	.670		0.13	1	.724		**4.31**	**1**	**.038**	**4D**	0.16	1	.686
Hab. div × Fragm.	0.19	1	.662		0.33	1	.567		0.05	1	.830		0.27	1	.607
Hab. div × Hab. am.	0.78	1	.379		<0.01	1	.960		0.28	1	.595		0.95	1	.330
Fragm. × Hab. am.	0.96	1	.327		0.75	1	0.386		0.56	1	.452		3.64	1	.056
Total variance explained			.094				.281				.451				.485

(Continues)

TABLE 1 (Continued)

	Richness				Evenness				LCBD				Abundance			
	Chisq	*df*	*p*-val		Chisq	*df*	*p*-val		Chisq	*df*	*p*-val		Chisq	*df*	*p*-val	
1,000 m BUFFER																
Summer																
Host plant	12.56	6	.051		11.96	6	.063		**41.43**	**6**	**<.001**		**29.58**	**6**	**<.001**	
Elevation	0.01	1	.920		0.13	1	.716		**11.73**	**1**	**.001**	**4I**	0.47	1	.494	
Precipitation	**8.30**	**1**	**.004**	**4J**	**5.40**	**1**	**.020**		1.99	1	.158		0.32	1	.569	
Habitat diversity	0.54	1	.464		0.03	1	.857		0.63	1	.428		0.99	1	.319	
Fragmentation	0.07	1	.795		<0.01	1	.984		0.02	1	.883		0.15	1	.695	
Habitat amount	0.33	1	.566		0.30	1	.585		0.61	1	.435		0.03	1	.872	
Hab. div × Fragm.	0.46	1	.499		0.83	1	.361		0.52	1	.469		0.11	1	.745	
Hab. div × Hab. am.	3.84	1	.050		2.32	1	.128		0.52	1	.471		0.09	1	.764	
Fragm. × Hab. am.	2.05	1	.152		1.53	1	.216		0.89	1	.346		0.15	1	.694	
Total variance explained			.321				.262				.415				.184	
Winter																
Host plant	6.18	7	.519		**22.78**	**7**	**.002**		**86.65**	**7**	**<.001**		**76.92**	**7**	**<.001**	
Elevation	**4.06**	**1**	**.044**	**4G**	**6.43**	**1**	**.011**	**4H**	0.11	1	.738		0.02	1	.886	
Precipitation	1.09	1	.296		1.16	1	.281		0.08	1	.772		0.61	1	.433	
Habitat diversity	0.02	1	.891		0.79	1	.373		1.06	1	.302		1.54	1	.215	
Fragmentation	1.04	1	.307		1.34	1	.246		2.81	1	.093		**4.13**	**1**	**.042**	**4K**
Habitat amount	1.17	1	.279		0.01	1	.942		**7.18**	**1**	**.007**	**4L**	0.60	1	.438	
Hab. div × Fragm.	0.02	1	.878		0.18	1	.673		0.01	1	.919		0.13	1	.719	
Hab. div × Hab. am.	0.09	1	.766		0.03	1	.866		0.07	1	.794		1.16	1	.281	
Fragm. × Hab. am.	0.77	1	.381		0.13	1	.717		0.12	1	.733		1.15	1	.283	
Total variance explained			.097				.286				.442				.503	

Bold values are those that are lower than 0.05.

between 500 and 1,000 m.a.s.l., although fragmentation values were larger at elevations below 500 than above 1,000 m.a.s.l. The amount of habitat increased steeply from 0 to 400 m.a.s.l and then a plateau was reached, whereas habitat diversity decreased gradually with elevation. These two opposing effects probably reflect the dominance of natural forests at higher elevations, leading to a larger proportion of habitat available, but to a lower habitat diversity (or evenness).

In the multi-scale analysis, the deviance explained by the different landscape features varied greatly among landscape features and diversity metrics, and none of the buffer sizes used explained model variation greater than the others (Appendix S12). Based on this result, it was not possible to identify the buffer at which spatial effects were strongest and models were therefore built using landscape data from two different buffer sizes that may explain short and long-scale effects, 300 and 1,000 m, respectively (Table 1, but see Appendix S13 for analyses using the other three buffer sizes).

4 | Plant effects on insect diversity and abundance

The plant from which insects were collected was the variable with the strongest effect on insect evenness, beta diversity and abundance (Table 1, Figure 2). The plants *H. ambavilla* and *L. camara* had particularly large values of thrips evenness, *D. ficulnea*, *H. ambavilla* and *Ipomoea* sp. had the most different thrips assemblages (i.e. largest values of LCBD), whereas *D. ficulnea*, *Ipomoea* sp. and *S. mauritianum* were the plants from where the largest insect abundances were obtained. Species richness was only marginally affected by the plant in summer when using landscape data in the buffer of 1,000 m (Table 1). *L. camara* was the plant that contained the richest insect assemblage.

5 | Effect of elevation and rainfall on insect diversity and abundance

Of the different environmental variables considered, only rainfall was retained in the models because temperature was strongly correlated ($R < 95\%$) with elevation (Appendix S5). In agreement with our hypotheses, elevation had a negative effect on insect alpha and beta diversity (Figure 3; Table 1). When models were built with data from 300 m buffers, the effect was observed in winter on species evenness (Table 1; Figure 4a). This effect was also observed on richness and evenness in winter when models were built with data from 1,000 m buffers (Table 1; Figure 4g and h). Elevation correlated negatively with beta diversity (LCBD) in summer in models using data from both 300 and 1,000 m buffers (Table 1, Figure 4b and i). These results suggest that in Reunion Island thrips are richer, and the different species are more evenly represented and unique in lowlands. Rainfall had a negative effect on thrips species richness and evenness in the models built with landscape data from both 300 and 1,000 m buffers. This effect, however, was only observed in summer (Table 1; Figure 4c and j).

6 | Effect of fragmentation, habitat diversity and habitat amount on insect diversity and abundance

In winter, when including landscape data from both 300 or 1,000 m buffers, beta diversity and habitat amount related negatively (Table 1, Figure 4d and l). In the models built using data from 1,000 m buffers, insect abundance related positively with fragmentation in winter (Table 1, Figure 4k). In summer, and when using landscape data from 300 m buffers, for both species richness and evenness significant interactions between habitat amount and habitat diversity were found (Table 1). In both cases, interaction plots revealed that habitat amount had a positive effect on insect richness and evenness, but this effect was lost in areas of low habitat diversity (Figure 4e and f). This effect was not observed in winter.

7 | DISCUSSION

In agreement with our hypotheses, insect richness and evenness decreased with elevation, although the effect was only observed in winter. In summer, accumulated monthly rainfall was the abiotic variable that dominated over thrips alpha diversity with a negative effect too. These contrasting seasonal effects may reflect the environmental conditions that dominate these two seasons. In the Indian Ocean, summer is rainy, warm and often with violent cyclones that may wash away adult thrips, an impact already observed by Boissot, Reynaud, and Letourmy (1998) in this same area. In winter, however, low temperatures are likely to be the most important factor reducing alpha diversity at high elevations. As in many other insects in the tropics (Kishimoto-Yamada & Itioka, 2015), abiotic variables are therefore important factors determining diversity patterns in our study. Insect beta diversity also decreased with elevation in summer, a result that we hypothesized. This effect is unlikely to be caused by the presence or absence of few influential species, or sampling effects because the effect was found in areas with particularly large insect richness. It is surprising, however, that in a hotspot of diversity like Reunion, lowland habitats that are dominated by invasive plant species (like *L. camara*) and urbanization (Strasberg et al., 2005) host the most diverse and unique thrips assemblages. Many studies have already reported similar correlations between human populations and diversity (Luck, 2007) probably because human populations concentrate in areas with larger annual energy budgets that lead to more benign conditions for organisms to thrive (Gaston, 2005). Thrips fauna in the Indian Ocean is poorly studied, and it is therefore difficult to know whether these lowland thrips assemblages are dominated by exotic species that reached the island together with exotic plants, or by native insects. The former is a likely situation because invasive insects often reach new territories together

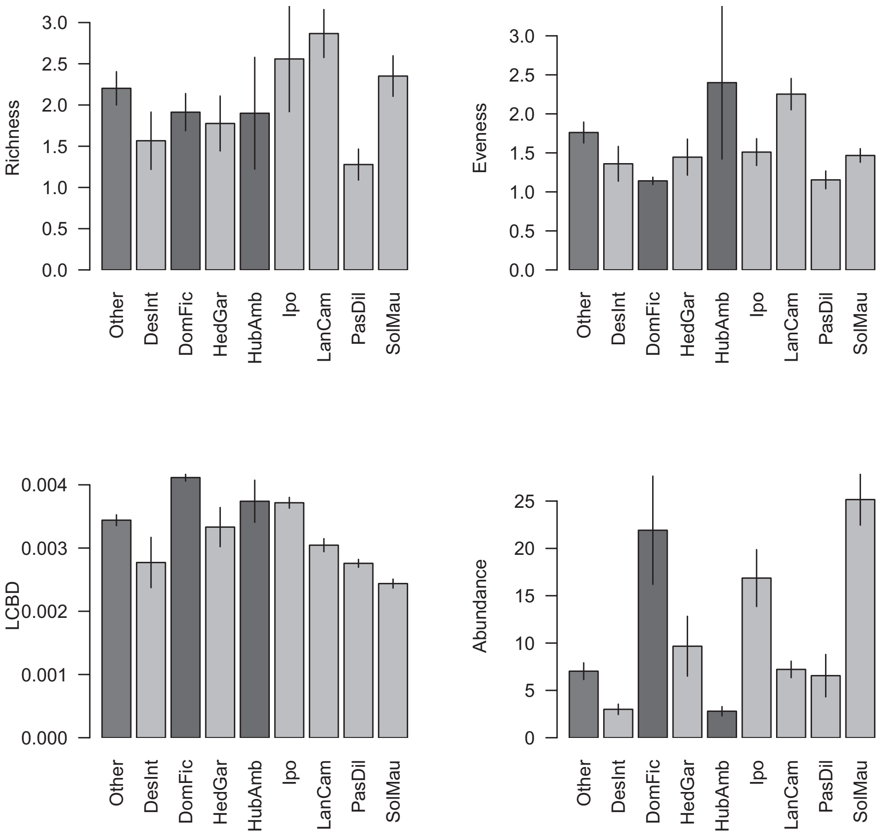


FIGURE 2 Thrips diversity and abundance on the different plants studied. Alpha diversity is expressed as species richness and evenness (Simpson index), and beta diversity as the local contribution to beta diversity metric (LCBD). Dark and light grey bars represent endemic and exotic plants, respectively. The category "others" includes plants that were sampled less than five times during the study. Abbreviations represent the following plants: *Desmodium intortum* (DesInt), *Dombeya ficulnea* (DomFic), *Hedychium gardnerianum* (HedGar), *Hubertia ambavilla* (HubAmb), *Ipomoea* spp. (Ipo), *Lantana camara* (LanCam), *Paspalum dilatatum* (PasDil) and *Solanum mauritianum* (SolMau). Details on the plants sampled can be found in Appendix S06.

with their host plants (Liebhold, Brockerhoff, Garrett, Parke, & Britton, 2012). Knowing where native thrips are located would be particularly useful to identify hotspots of endemic diversity within the island (Socolar et al., 2016), and to unveil the role that invasive plants played in determining insect diversity patterns.

Thrips diversity was influenced by landscape features, when extracting landscape information from 300 and 1,000 m buffers. This suggests that these minute insects are able to disperse large distances as has already been reported for some species (Mound, 2005). In summer, both species richness and evenness were significantly related to habitat amount, but this effect depended on habitat diversity. The relationship between habitat amount and insect diversity was positive in highly diverse landscapes, but this effect was offset in areas of low diversity. In agreement with our hypotheses, the negative effect of habitat loss on insect diversity may be thus exacerbated in low diversity habitats. In a recent study, Corcos et al. (2018) explored whether the effect of temperature on the diversity of a range of different insect guilds was modulated by habitat diversity along elevational gradients. These authors found contrasting effects on the different insect guilds studied, but the interaction between landscape and elevation was never significant. As done by these authors, our measure of habitat diversity was based on the proportion of different habitat types within the buffer, also known as land cover diversity. This measure mainly expresses topographic heterogeneity, and a meta-analysis by Stein et al. (2014) revealed its strong effect on the richness of terrestrial plants and animals. In this meta-analysis, however, plant diversity and vegetation complexity also had strong impacts on diversity. Studies with cavity-nesting bees and wasps (Staab et al., 2016), and with insect predators (Vehviläinen, Koricheva, & Ruohomäki, 2008), for instance, revealed that diversity at the plant layer can even cascade up to higher trophic levels, and that the effect may depend on plant composition and on phylogenetic diversity [reviewed in (Moreira, Abdala-Roberts, Rasmann, Castagneyrol, & Mooney, 2015)]. It would be very interesting to obtain more detailed information from our study sites, and to explore how other habitat diversity measures affect the results obtained. Relative to lowlands, highlands in Reunion had the lowest habitat diversity values because these areas are uniformly dominated by natural forests. These habitats, however, are likely to be far more diverse in terms of plant richness so the results observed here may be reversed. Going deeper into the diversity of our study sites, for example exploring plant diversity, may also help to understand the unexpected negative effect that habitat amount had on beta diversity. Exploring the effect of plant diversity may require expertise in plant taxonomy and intense fieldwork, and using highly-resolved landscape layers is likely to be the most feasible technique in areas where plant taxonomy is not fully resolved, or fieldwork is challenging. The method used here can therefore be useful to compare our results with other animal taxa because few studies have integrated a suite of landscape variables to identify and isolate the factors that drive alpha and beta diversity changes along environmental gradients (Mori et al., 2018).

There is a long and unsolved debate over whether fragmentation has a positive or negative effect on diversity (Fahrig, 2019; Fletcher et al., 2018), and on whether negative effects are mostly

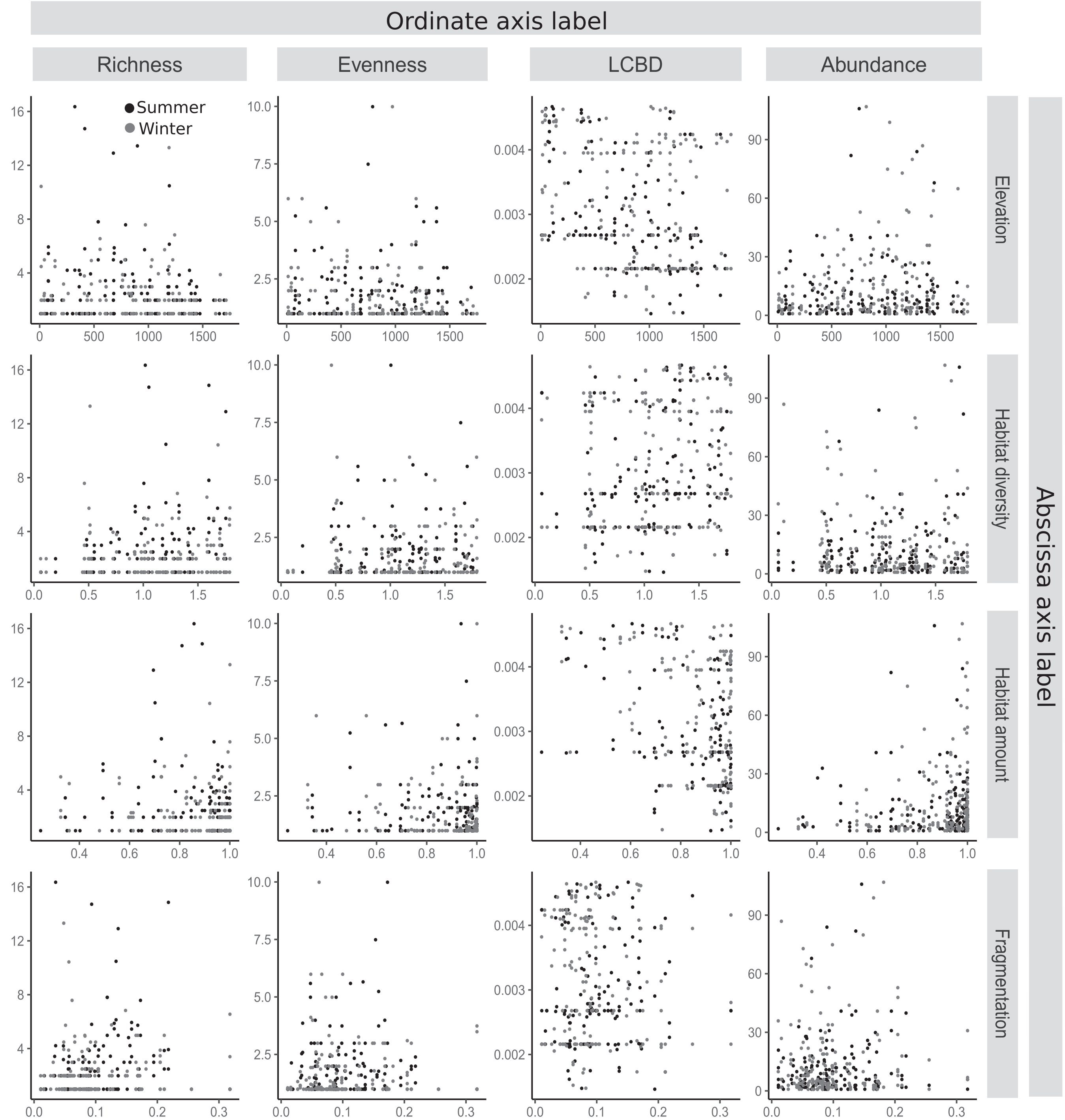
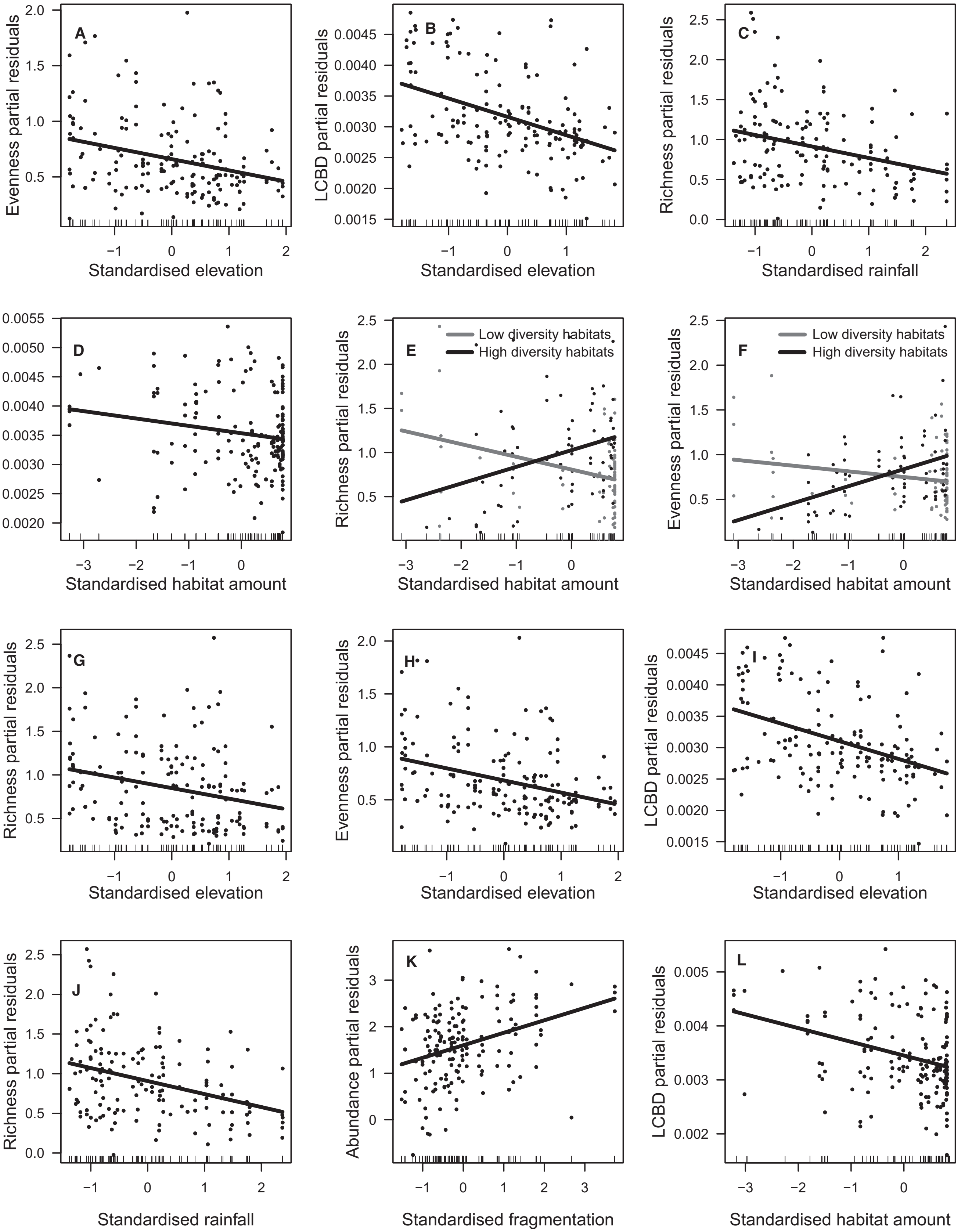


FIGURE 3 Scatterplots showing the relationship between elevation and landscape features with the different metrics of thrips diversity and abundance. Alpha diversity is expressed as species richness and evenness (Simpson index), and beta diversity as the local contribution to beta diversity metric (LCBD). Landscape features are estimated using 1000 m buffers. Black and grey dots represent values estimated in summer and winter, respectively.

due to a reduction in the amount of habitat available. In our study, fragmentation had a positive effect on thrips total abundance in winter, but overall this variable had little influence on diversity estimates. Relative to habitat amount or habitat diversity, fragmentation is therefore a minor driver of thrips diversity in Reunion Island. In addition, if the models built are simplified and habitat amount removed (results not shown), fragmentation becomes a significant explanatory variable for diversity in some cases, which suggests that habitat amount may sometimes be the reason behind significant effects of fragmentation on diversity. Altogether, these results provide additional support to the habitat amount hypothesis (Fahrig, 2013; Watling et al., 2020), although more experimental work, and not just observational studies is needed to confirm this tendency over a range of taxa and ecosystem types.



Our diversity estimates were based on adult thrips collected from flowers, but their larvae are known to predate on other insects, or to feed on a variety of plants and fungi, and then to disperse as adults to feed on pollen or to mate (Mound, 2005). Thrips are easy to sample and have a large functional and taxonomic diversity, which makes this insect order a good model to study diversity patterns. Even if some

FIGURE 4 Plots showing partial residuals from the models on the effect of elevation, rainfall and landscape variables on thrips diversity and abundance when extracting landscape information from 300m (A-F) and 1000m (G-L) buffers. Only significant effects are plotted (see Table 1). The fitted lines are estimated from the linear mixed effects models, the points represent the partial residuals from the models, based on the *visreg* package in R, and the rugplot is shown along the abscissa axis. To fulfil model assumptions all analyses are performed after standardising all predictor continuous variables. A. The effect of elevation on thrips evenness in winter; B. The effect of elevation on thrips beta diversity (LCBD) in summer; C. The effect of accumulated rainfall on thrips species richness in summer; D. The effect of habitat amount on thrips beta diversity (LCBD) in winter. The effect of habitat amount and its interactions with habitat diversity on thrips species richness (E) and evenness (F) in summer. The interaction represents high (dark grey) and low (light grey) values of habitat diversity; The effect of elevation on thrips species richness (G) and evenness (H) in winter; I. The effect of elevation on thrips beta diversity (LCBD) in summer; J. The effect of accumulated rainfall on thrips species richness in summer; K. The effect of fragmentation on thrips abundance in winter; L. The effect of habitat amount on thrips beta diversity (LCBD) in winter. For both the models built using landscape data from 300 and 1000m, the significant effect of accumulated rainfall on thrips evenness in summer has been omitted as the relationship is very similar to that with species richness. Raw data ranges can be seen in Figure 3.

thrips species are known to be pollinators, this group is not usually considered as a pollinator guild, and most species are considered as passive dispersers with weak interactions with flowers as adults (Mound, 2005). The multivariate analysis revealed that plants had a strong effect on thrips composition, thereby suggesting that this group can also be useful to study insect–plant interaction networks. For example, the newly found species, *T. reunionensis* (Goldarazena et al., 2020) was associated with the endemic plant *Dombeya ficulnea*, but only in winter when this plant was in full bloom.

One reason why the elevational diversity gradient in herbivores is not fully understood is that it depends on several interacting factors. Several environmental variables are hypothesized to be the driving force, but also their indirect effect via primary productivity and competitive interactions (Kreft & Jetz, 2007; Vetaas, Paudel, & Christensen, 2019). Many hypotheses like the mid domain effect or the water-energy dynamics have been proposed. As we show here, finding general patterns may be challenging if habitat disturbance by human populations is not taken into account. Reunion is a small island in which the landscape changes dramatically with elevation because human settlements concentrate in lowlands, a large national park covers high elevation areas, and agricultural lands are found in between with strong differences between the east and the west of the island (Strasberg et al., 2005). Even if some landscape variables like habitat amount were collinear with elevation, elevation impacted the different habitat features measured differently. This situation offered a unique opportunity to explore how, in addition to elevation, landscape features affect diversity patterns. Island diversity, especially in highly populated and remote islands like Reunion, is particularly threatened by habitat loss and invasive species. Given the disproportionate diversity that tropical islands host, understanding how diversity varies across space and time in these areas can help designing future conservation efforts (Russell & Kueffer, 2019). Many thrips species are pests, and studying the diversity of this taxa can also help to understand their spatio-temporal dynamics and allow the design of agricultural landscapes in which pest outbreaks are less likely.

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DATA AVAILABILITY STATEMENT

The data sets generated during and/or analysed during the current study are available at Dryad doi:10.5061/dryad.18931zcvk. R code used to analyse data can be accessed from the corresponding author on reasonable request.

ORCID

Enric Frago https://orcid.org/0000-0001-8817-1303

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BIOSKETCH

Niry. T. Dianzinga is a PhD student of the University of Reunion based at CIRAD/3P interested in the ecology and taxonomy of thrips and on insect community ecology in general. This PhD was developed in the team led by Enric Frago who is an insect ecologist interested in insect community ecology, insect-plant-microbe interactions and biocontrol.

Author contributions: N.T.D. and E.F conceived and designed the study; Author contributions: N.T.D., M-L.M. and E.F. collected insects; N.T.D identified insects; J.S. performed the molecular laboratory work; N.T.D. and E.F analysed data and wrote the manuscript with comments from all co-authors.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Appendix figure and table legends

S1. List of study sites established in this study, with details on the season each site was sampled, its elevation and geographic coordinates, the municipality to which it belongs, and the plants that were sampled.

S2. Extended methods used to identify thrips morphologically, and via barcode. Details on how the phylogenetic tree was built are also provided.

S3. List and bibliographic details of the different R packages used.

S4. Scaterplots showing the relationship between elevation and the different metrics of thrips diversity and abundance. Black and grey dots represent values estimated in summer and winter, respectively.

S5. Matrix showing R values of the Spearman correlation between elevation and environmental variables including mean, maximum, minimum and accumulated annual rainfall.

S6. List of plants sampled in this study including their scientific name, the family they belong, whether they are exotic or native, and the number of times each plant was sampled. Plants in boldface are those that were sampled at leas five times during the whole study. (*) Number of times at least one individual thrips was obtained during a plant sampling event.

S7. List of thrips species found in this study, the family they belong and the total number of individuals obtained.

S8. Variables influencing thrips community structure in summer and winter represented with a non-metric multidimensional scaling (NMDS) analysis.

S9. Phylogenetic tree of thrips specimens obtained in this study and from NCBI-GenBank (in grey). The tree was built with the maximum likelihood method. Percent bootsrap values are shown in nodes.

S10. Proportion of the different habitat types along elevation in the studied sites when using buffers of either 300m or 1000m of radius. Within each transect, the different sites were grouped into categories of 200m of elevation so that mean (± SE) values for the seven transects can be plotted. Habitat types with an average of less than 5% coverage within buffers are not included. Habitat types included in the figure are the following: sugar cane (CanSuc), forest (Forest), grassland (Prairie), savannah (Savane), orchards (Verger) and urban habitats (ZonUrb).

S11. Proportion of the different landscape features along elevation in the studied sites when using buffers of either 300m or 1000m of radius. Within each transect, the different sites were grouped into categories of 200m of elevation so that mean (± SE) values for the seven transects can be plotted.

S12. Multi-scale analysis showing the proportion of deviance explained by the different diversity and abundance estimates and landscape features along increasing buffer sizes (i.e. 100m, 300m, 600m, 1000m and 3000m of radius). Extended methods and results are presented together with a graphical representation. In the plot, the solid dot represents the only variable with a significant effect after correction for multiple comparisons using the false discovery rate approach.

S13. Table showing the effect of elevation, plant of collection, accumulated rainfall, habitat diversity, fragmentation, habitat amount and pairwise interactions between landscape features on thrips alpha diversity (species richness and evenness), beta diversity (local contribution to beta diversity, LCBD) and abundance when extracting landscape data from buffers of 100m, 600m and 3000m of radius. A different mixed effects model was built for each buffer size, diversity and abundance estimates in summer and winter. Significant *P*-values are presented in boldface type. The total amount of variance explained by each model is also shown.

Appendix 1

transect	site	season	location	ele	lat	lon	host plant
transect1	site1	summer	Sainte-Marie	22.752	-20.895	55.540	–
transect1	site2	summer	Sainte-Marie	204.130	-20.922	55.538	*Cynodon dactylon*
transect1	site3	summer	Sainte-Marie	392.456	-20.939	55.536	*Conyza sumatrensis*
transect1	site4	summer	Sainte-Marie	554.503	-20.947	55.535	*Conyza sumatrensis* *Eleusine aegyptica*
transect1	site5	summer	Sainte-Marie	781.850	-20.956	55.535	*Solanum mauritianum* *Paspalum dilatatum* *Lantana camara*
transect1	site6	summer	Sainte-Marie	1079.673	-20.969	55.528	*Lantana camara* *Paspalum dilatatum* *Solanum mauritianum* *Tibuchina grandifolia*
transect1	site7	summer	Grand Bras Sec	1228.010	-20.976	55.520	*Hedychium gardnerianum* *Paspalum dilatatum*
transect1	site8	summer	Grand Bras Sec	1376.547	-20.983	55.514	*Paspalum dilatatum* *Bertiera rufa* *Chassalia corallioides* *Hedychium gardnerianum* *Humbertia ambavilla* *Solanum mauritianum*
transect1	site1	winter	Sainte-Marie	22.752	-20.895	55.540	–
transect1	site2	winter	Sainte-Marie	204.130	-20.922	55.538	*Solanum mauritianum*
transect1	site3	winter	Sainte-Marie	392.456	-20.939	55.536	*Ipomoea* sp. *Solanum mauritianum*
transect1	site4	winter	Sainte-Marie	554.503	-20.947	55.535	*Lantana camara* *Solanum mauritianum* *Euphorbia fulgens*
transect1	site5	winter	Sainte-Marie	781.850	-20.956	55.535	*Solanum mauritianum* *Lantana camara*
transect1	site6	winter	Sainte-Marie	1090.587	-20.970	55.528	*Dombeya ficulnea* *Lantana camara* *Solanum mauritianum*
transect1	site7	winter	Gras Bras Sec	1335.396	-20.983	55.516	*Dombeya ficulnea* *Solanum mauritianum*
transect1	site8	winter	Gras Bras Sec	1377.092	-20.984	55.514	*Solanum mauritianum* *Dombeya ficulnea*
transect2	site1	summer	La Possession	17.098	-20.923	55.345	*Panicum maximum*
transect2	site2	summer	La Possession	114.663	-20.928	55.345	*Panicum maximum* *Chloris barbata* *Tribulus cistoides*
transect2	site3	summer	La Possession	413.849	-20.927	55.365	*Lantana camara* *Solanum mauritianum* *Paspalum dilatatum*
transect2	site4	summer	La Possession	617.295	-20.928	55.393	*Lantana camara* *Solanum mauritianum*
transect2	site5	summer	Saint-Denis	864.352	-20.933	55.400	*Lantana camara* *Solanum mauritianum* *Ipomoea* sp.
transect2	site6	summer	Plaines d'Affouches	1060.648	-20.941	55.406	*Lantana camara* *Solanum mauritianum*
transect2	site7	summer	Plaines d'Affouches	1187.042	-20.952	55.405	*Solanum mauritianum* *Lantana camara* *Verbena bonariensis*
transect2	site8	summer	Plaines d'Affouches	1325.634	-20.958	55.407	*Lantana camara* *Humbertia ambavilla*
transect2	site1	winter	La Possession	12.731	-20.923	55.345	*Malvastrum coromandelianum* *Sonchus oleraceus*
transect2	site2	winter	La Possession	261.065	-20.928	55.355	*Rhus longipes*
transect2	site3	winter	La Possession	474.538	-20.924	55.370	*Rhus longipes* *Solanum mauritianum* *Ipomoea* sp. *Lantana camara*
transect2	site4	winter	Saint-Denis	858.400	-20.932	55.400	*Lantana camara* *Ipomoea* sp.

							Crotalaria berteroana *Solanum mauritianum*
transect2	site5	winter	Saint-Denis	971.486	-20.939	55.401	*Lantana camara* *Rhus longipes* *Solanum mauritianum* *Ipomoea* sp.
transect2	site6	winter	Plaines d’Affouches	1148.329	-20.948	55.403	*Nuxia verticillata* *Dombeya ficulnea* *Solanum mauritianum* *Dodonaea viscosa* *Erica reunionensis* *Weinmannia trinctoria*
transect2	site7	winter	Plaines d’Affouches	1189.068	-20.951	55.402	*Nuxia verticillata* *Dombeya ficulnea* *Solanum mauritianum* *Hypericum lanceolatum* *Humbertia ambavilla*
transect2	site8	winter	Plaines d’Affouches	1286.783	-20.959	55.404	*Solanum mauritianum* *Dombeya ficulnea*
transect3	site1	summer	Saint-Paul	65.550	-21.016	55.269	*Guazuma ulmifolia*
transect3	site2	summer	Saint-Paul	117.893	-21.019	55.266	*Caesalpinia pulcherrima*
transect3	site3	summer	Saint-Paul	362.625	-21.034	55.279	*Solanum mauritianum* *Lantana camara* *Antigonon leptopus*
transect3	site4	summer	Saint-Paul	676.997	-21.040	55.303	*Lantana camara* *Duranta repens* *Ipomoea* sp. *Setaria pumila* *Amaranthus spinosus*
transect3	site5	summer	Saint-Paul	853.707	-21.040	55.319	*Solanum mauritianum* *Ipomoea* sp. *Lantana camara*
transect3	site6	summer	Saint-Paul	990.548	-21.044	55.327	*Lantana camara* *Hydrangea macrophylla* *Tibouchina urvilleana* *Ligustrum lucidum* *Solanum mauritianum*
transect3	site7	summer	Maïdo	1260.094	-21.048	55.343	*Lantana camara* *Solanum mauritianum*
transect3	site8	summer	Maïdo	1472.961	-21.054	55.353	*Solanum mauritianum* *Lantana camara* *Humbertia ambavilla*
transect3	site9	summer	Maïdo	1695.784	-21.056	55.364	*Solanum mauritianum* *Panicum maximum*
transect3	site1	winter	Saint-Paul	12.286	-20.994	55.310	*Solanum mauritianum* *Litchi sinensis*
transect3	site2	winter	Saint-Paul	117.893	-21.019	55.266	−
transect3	site3	winter	Saint-Paul	387.133	-21.034	55.282	*Schinus terebinthifolia* *Lantana camara*
transect3	site4	winter	Saint-Paul	554.264	-21.039	55.292	*Solanum mauritianum* *Ipomoea* sp. *Lantana camara*
transect3	site5	winter	Saint-Paul	761.532	-21.039	55.312	*Solanum mauritianum* *Ipomoea* sp. *Lantana camara* *Hedychium gardnerianum*
transect3	site6	winter	Saint-Paul	931.303	-21.041	55.323	*Solanum mauritianum* *Lantana camara*
transect3	site7	winter	Maïdo	1209.577	-21.048	55.340	*Solanum mauritianum* *Lantana camara* *Hedychium gardnerianum*
transect3	site8	winter	Maïdo	1434.916	-21.052	55.352	*Solanum mauritianum* *Lantana camara*
transect3	site9	winter	Maïdo	1749.060	-21.060	55.366	*Acacia heterophylla* *Solanum mauritianum* *Dombeya ficulnea*
transect4	site1	summer	Pierrefonds	46.515	-21.314	55.431	*Bougainvillea* sp. *Psiadia retusa* *Heliotropium indicum*
transect4	site2	summer	Saint-Pierre	119.247	-21.290	55.453	*Lantana camara*
transect4	site3	summer	Saint-Louis	322.445	-21.217	55.454	*Solanum mauritianum* *Lantana camara*

transect4	site4	summer	Saint-Louis	466.981	-21.188	55.451	*Lantana camara*
transect4	site5	summer	Saint-Louis	680.208	-21.181	55.453	*Solanum mauritianum* *Lantana camara*
transect4	site6	summer	Cilaos	831.105	-21.176	55.459	*Solanum mauritianum* *Lantana camara*
transect4	site7	summer	Cilaos	935.982	-21.172	55.463	*Solanum mauritianum*
transect4	site8	summer	Cilaos	1180.330	-21.142	55.469	*Solanum mauritianum* *Russelia equisetiformis* *Bougainvillea* sp. *Lantana camara* *Ligustrum lucidum*
transect4	site9	summer	Bras Sec	1392.527	-21.136	55.496	*Solanum mauritianum* *Lantana camara*
transect4	site1	winter	Pierrefonds	39.307	-21.320	55.444	*Psiadia retusa* *Coccinia grandis* *Schinus terebinthifolia* *Ricinus communis*
transect4	site2	winter	Saint-Pierre	140.236	-21.276	55.454	*Parthenium hysterophorus* *Solanum mauritianum* *Lantana camara* *Erigeron karvinskianus* *Solanum torvum*
transect4	site3	winter	Saint-Louis	410.131	-21.189	55.450	*Solanum mauritianum* *Lantana camara*
transect4	site4	winter	Saint-Louis	601.915	-21.183	55.453	*Solanum mauritianum* *Lantana camara*
transect4	site5	winter	Cilaos	842.205	-21.176	55.456	*Sorghum arundinaceum* *Lantana camara* *Desmodium intortum* *Solanum mauritianum*
transect4	site6	winter	Cilaos	1023.815	-21.154	55.468	*Lantana camara* *Desmodium intortum* *Solanum mauritianum* *Ipomoea* sp.
transect4	site7	winter	Cilaos	1223.237	-21.132	55.474	*Lantana camara* *Desmodium intortum* *Solanum mauritianum* *Datura* sp. *Erigeron karvinskianus* *Eriobotrya japonica* *Leucaena leucocephala*
transect4	site8	winter	Bras Sec	1398.267	-21.126	55.492	*Lantana camara* *Desmodium intortum* *Solanum mauritianum* *Nuxia verticillata*
transect5	site1	summer	Ravine des Cafres	79.178	-21.350	55.510	*Lantana camara* *Thevetia peruviana* *Tagetes patula*
transect5	site2	summer	Saint-Pierre	104.342	-21.347	55.511	*Bougainvillea* sp.
transect5	site3	summer	Saint-Pierre	372.851	-21.330	55.528	*Bougainvillea* sp. *Lantana camara* *Allamanda blanchetti*
transect5	site4	summer	Saint-Pierre	537.295	-21.315	55.536	*Tagetes patula* *Lantana camara* *Ipomoea* sp. *Tibuchina grandifolia*
transect5	site5	summer	Le Tampon	752.350	-21.284	55.544	*Solanum mauritianum* *Lantana camara*
transect5	site6	summer	Le Tampon	963.952	-21.283	55.558	*Solanum mauritianum* *Lantana camara* *Tropaeolum majus*
transect5	site7	summer	Le Tampon	1177.904	-21.276	55.572	*Solanum mauritianum* *Lantana camara*
transect5	site8	summer	Le Tampon	1429.054	-21.273	55.588	*Solanum mauritianum* *Hypericum lanceolatum*
transect5	site9	summer	Notre Dame de la Paix	1600.098	-21.270	55.598	*Solanum mauritianum*
transect5	site1	winter	Ravine des Cafres	42.168	-21.354	55.510	*Lantana camara* *Abutilon indicum* *Schinus terebinthifolia*
transect5	site2	winter	Saint-Pierre	104.342	-21.347	55.511	*Lantana camara*

transect5	site3	winter	Saint-Pierre	438.481	-21.325	55.532	*Solanum mauritianum* *Lantana camara* *Cajanus cajan*
transect5	site4	winter	Le Tampon	610.018	-21.293	55.534	*Solanum mauritianum* *Cajanus cajan* *Ipomoea* sp.
transect5	site5	winter	Le Tampon	817.169	-21.290	55.552	*Solanum mauritianum* *Pennisetum purpureum*
transect5	site6	winter	Le Tampon	1032.628	-21.281	55.563	*Solanum mauritianum* *Lantana camara* *Holmskioldia sanguinea* *Paspalum paniculum* *Paspalum urvillei*
transect5	site7	winter	Le Tampon	1240.716	-21.275	55.576	*Solanum mauritianum* *Lantana camara* *Pennisetum purpureum* *Ulex europaeus*
transect5	site8	winter	Le Tampon	1430.600	-21.273	55.589	*Solanum mauritianum* *Lantana camara* *Acacia mearnsii* *Ulex europaeus*
transect5	site9	winter	Notre Dame de la Paix	1713.896	-21.263	55.600	*Ulex europaeus* *Solanum mauritianum* *Nuxia verticillata*
transect6	site1	summer	Saint-Benoît	7.614	-21.045	55.725	*Lantana camara*
transect6	site2	summer	Saint-Benoît	252.181	-21.080	55.695	*Solanum mauritianum* *Paspalum dilatatum*
transect6	site3	summer	Saint-Benoît	500.010	-21.100	55.672	*Solanum mauritianum* *Lantana camara* *Impatiens flaccida*
transect6	site4	summer	Saint-Benoît	604.534	-21.103	55.671	*Lantana camara* *Paspalum dilatatum* *Verbena bonariensis*
transect6	site5	summer	La Plaine des Palmistes	1012.703	-21.134	55.630	*Lantana camara* *Paspalum dilatatum* *Crocosmia ×crocosmiiflora*
transect6	site6	summer	La Plaine des Palmistes	1283.185	-21.155	55.602	*Lantana camara* *Solanum mauritianum* *Verbena bonariensis*
transect6	site7	summer	La Plaine des Palmistes	1415.901	-21.160	55.597	*Solanum mauritianum* *Paspalum dilatatum*
transect6	site8	summer	La Plaine des Palmistes	1617.894	-21.166	55.589	*Solanum mauritianum*
transect6	site1	winter	Saint-Benoît	16.538	-21.053	55.722	*Solanum mauritianum* *Ipomoea* sp. *Ageratum conyzoides*
transect6	site2	winter	Saint-Benoît	218.844	-21.076	55.699	*Solanum mauritianum* *Ipomoea* sp. *Ageratum conyzoides*
transect6	site3	winter	Saint-Benoît	335.975	-21.087	55.690	*Solanum mauritianum* *Ageratum conyzoides*
transect6	site4	winter	Saint-Benoît	610.312	-21.104	55.671	*Solanum mauritianum* *Ipomoea* sp.
transect6	site5	winter	La Plaine des Palmistes	882.647	-21.116	55.649	*Solanum mauritianum* *Ipomoea* sp. *Dombeya ficulnea* *Hedychium gardnerianum*
transect6	site6	winter	La Plaine des Palmistes	1013.245	-21.134	55.630	*Solanum mauritianum* *Hedychium gardnerianum* *Ipomoea* sp. *Ficus rubra* *Digitalis purpurea*
transect6	site7	winter	La Plaine des Palmistes	1283.185	-21.155	55.602	*Solanum mauritianum* *Hedychium gardnerianum* *Ipomoea* sp. *Dombeya ficulnea*
transect6	site8	winter	La Plaine des Palmistes	1415.901	-21.160	55.597	*Dombeya ficulnea*
transect6	site9	winter	La Plaine des Palmistes	1617.894	-21.166	55.589	*Dombeya ficulnea* *Solanum mauritianum* *Hedychium gardnerianum*
transect7	site1	summer	Saint-André	77.884	-20.960	55.663	*Lantana camara* *Solanum mauritianum*
transect7	site2	summer	Saint-André	302.119	-20.998	55.582	*Solanum mauritianum*

							Cyperus polystachyos *Hippobroma longiflora*
transect7	site3	summer	Salazie	452.238	-21.027	55.539	*Lantana camara* *Ipomoea* sp. *Allamanda cathartica* *Tibouchina grandifolia*
transect7	site4	summer	Salazie	684.471	-21.034	55.495	*Lantana camara* *Solanum mauritianum* *Brugmansia suaveolens*
transect7	site5	summer	Salazie	789.320	-21.032	55.489	*Lantana camara* *Solanum mauritianum* *Ligustrum lucidum*
transect7	site6	summer	Salazie	1012.536	-21.032	55.482	*Solanum mauritianum* *Lantana camara* *Ipomoea* sp.
transect7	site7	summer	Grand Îlet	1306.142	-21.040	55.464	*Solanum mauritianum* *Lantana camara* *Hydrangea macrophylla*
transect7	site8	summer	Grand Îlet	1442.571	-21.050	55.457	*Solanum mauritianum* *Lantana camara* *Cynorkis purpurascens*
transect7	site9	summer	Grand Îlet	1652.280	-21.055	55.455	*Solanum mauritianum* *Hubertia ambavilla*
transect7	site1	winter	Saint-André	117.749	-20.975	55.649	*Solanum mauritianum*
transect7	site2	winter	Salazie	433.925	-21.026	55.542	*Solanum mauritianum* *Ipomoea* sp.
transect7	site3	winter	Salazie	651.976	-21.033	55.526	*Solanum mauritianum* *Lantana camara* *Ageratum conyzoides*
transect7	site4	winter	Salazie	803.233	-21.032	55.489	*Solanum mauritianum* *Lantana camara*
transect7	site5	winter	Salazie	1098.365	-21.031	55.479	*Solanum mauritianum* *Ipomoea* sp. *Lantana camara*
transect7	site6	winter	Grand Îlet	1113.040	-21.030	55.477	–
transect7	site7	winter	Grand Îlet	1660.752	-21.053	55.454	*Solanum mauritianum* *Dombeya ficulnea*

Appendix S2

Morphological and molecular insect identification methods

Insects were stored in 10% Ethanol (containing a drop of liquid baseline per litter to break surface tension) during 10 days to prevent insects becoming too rigid and to facilitate morphological identification and then transferred into 70% ethanol and preserved at -20°C. Specimens were first sorted into morpho-species using a stereo-microscope and then, a subset of individuals identified to species level using a light microscope. To allow visualisation of internal structures, insects were immersed into lactic acid for two hours. All species were identified by N.T.D. using keys from (J.P. Bournier, 2000; Mound & Kibby, 1998). All thrips specimens are stored at the entomological collections of CIRAD-UMR PVBMT (CIRAD, Saint-Pierre, La Réunion) and at Centre de Biologie et de Gestion des Populations (CBGP-INRA, Montferrier-sur-Lez, France).

To ensure that morphological identifications were correct and to detect potential cryptic species, 223 specimens representing four known endemic species (*T. bourbonensis*, *T. candidus*, *T. quilicii* and *T. reunionensis* n. sp.) and six species with a global distribution (*T. parvispinus*, *T. florum*, *M. sjostedti*, *F. schultzei*, *H. pattersoni* and *H. gowdeyi*) were barcoded. These specimens were selected based on their abundance and the potential presence of cryptic species, as is the case of *F. schultzei* (Tyagi et al., 2017). To barcode these specimens, total genomic DNA was extracted from whole individuals using Qiagen DNeasy® 96 Blood & Tissue extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The Polymerase chain reaction (PCR) was performed to amplify about 750 base pairs (bp) from the 5'end of mtCOI gene using primer pair C_LepFolF and C_LepFolR, and a mixture of LepF1/LepR1 and LCO1490/HCO2198LepF1 and LepR1 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994; Hebert, Penton, Burns, Janzen, & Hallwachs, 2004). The PCR reaction was set in a 20 µl total volume containing 4 µl of genomic DNA, 0.25 µM of each primer and 10 µl of Taq polymerase (Type-it Multiplex PCR Master Mix, 2x) (Qiagen, Hilden, Germany). Cycling parameters were: 15 min at 95°C, 5 cycles of 30 s at 95°C, 30 s at 45°C, 60 s at 72°C, followed by 35 cycles of 30 s at 95°C, 60 s at 51°C, 60 s at 72°C and a final extension for 10 min at 72°C. PCR amplified products were sent to Macrogen Inc. (Amsterdam, Netherland) for purification and bidirectional sequencing using the Sanger method. The generated forward and reverse chromatograms were assembled to obtain the consensus sequences using *Geneious* software version 10.2.3 (Biomatters, New Zealand). 196 sequences for 10 described species were generated in the current study. Sequenced voucher specimens and their DNA were stored in the entomological collections of CIRAD-UMR PVBMT (CIRAD, Saint-Pierre, La Réunion) and CIRAD-UMR CBGP (CBGP-INRA, Montferrier-sur-Lez, France). Consensus sequences in *FASTA* format, were imported with 23 published barcode sequences pertaining to 10 species from NCBI-GenBank (https://www.ncbi.nlm.nih.gov/genbank/) into the program *MEGA* version 7.0.26 (Edgar, 2004) for alignment by amino acid. Once the open reading frame was determined, sequences were translated to amino acid, and then aligned in *MEGA* via *MUSCLE*. In order to obtain a sequence alignment of at least 500 bp, we eliminated the shortest sequences. Overall, 76 generated sequences and 18 published barcode sequences were included in the analyses. The model for nucleotide substitution was General Time Reversible using a discrete Gamma distribution with 5 rate categories and by assuming that a certain fraction of sites were evolutionary invariable (GTR+G+I). This model was assumed based on BIC (Bayesian Information Criterion) scores. Phylogenetic tree were built using the maximum likelihood method with 1000 replications as bootstrap values using the tool *RAxML-HPC* version 8.2.10 on *XSEDE* (Stamatakis, 2014) in *CIPRES Science Gateway* (Miller, Pfeiffer, & Schwartz, 2010).

Appendix 3.

In this work the following R packages have been used: *adespatial* (Dray et al., 2018), *ape* (Paradis & Schliep, 2019), *car* (Fox et al., 2018), *iNEXT* (Hsieh, Ma, & Chao, 2016), *lme4* (Bates, Maechler, Bolker, & Walker, 2014), *MuMIn* (Bartoń, 2019), *predictmeans* (Luo, Ganesh, & Koolaard, 2018), *raster (*Hijmans, 2019), *vegan* (Oksanen et al., 2017), *visreg* (Breheny & Burchett, 2017).

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Hsieh, T. C., Ma, K. H., & Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, *7*(12), 1451–1456. https://doi.org/10.1111/2041-210X.12613

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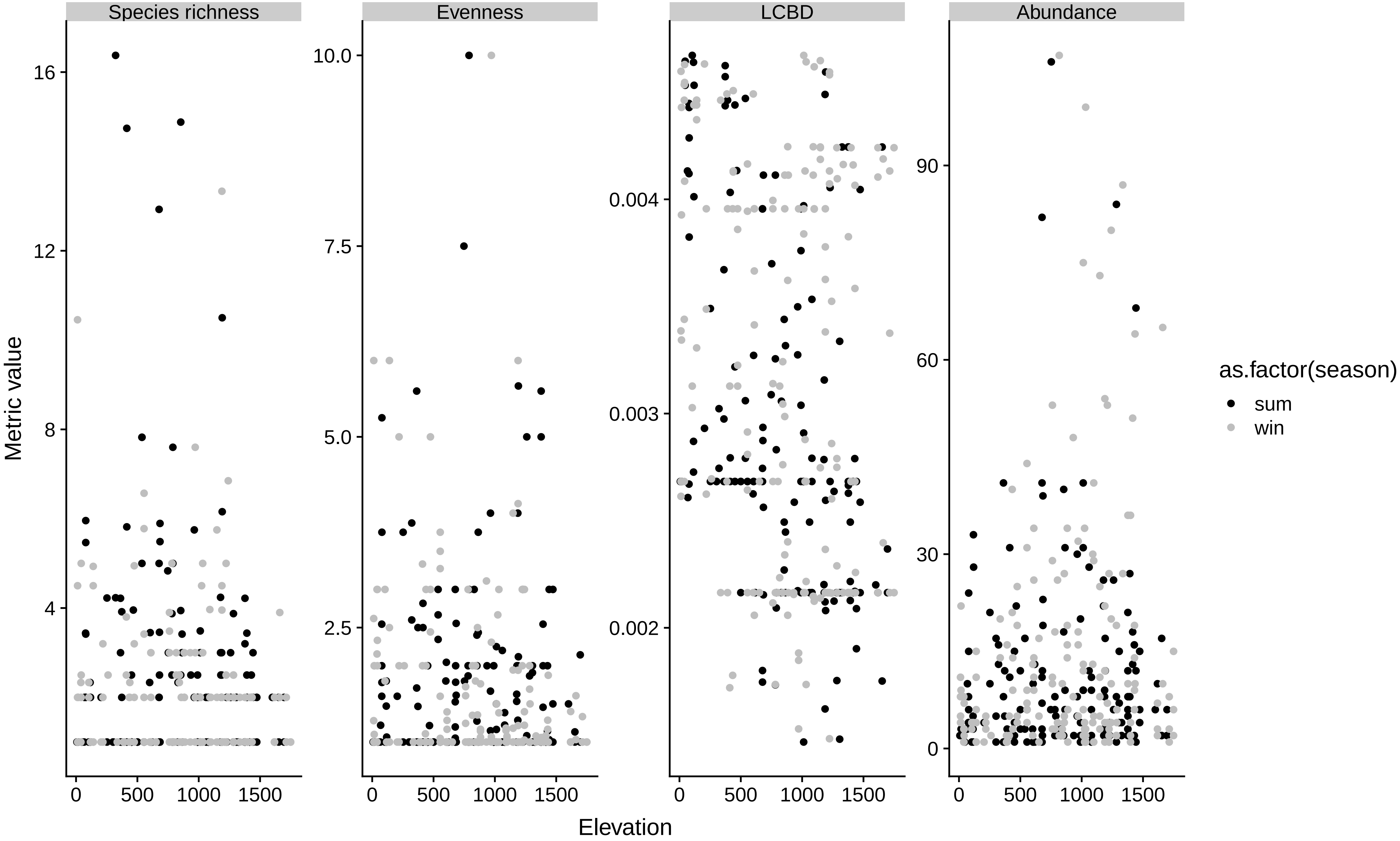
Appendix 4


Species richness
Evenness
LCBD
Abundance
Metric value
Elevation
16
12
8
4
10.0
7.5
5.0
2.5
0.004
0.003
0.002
90
60
30
0
0
500
1000
1500
as.factor(season)
● sum
● win

Appendix 5

	Elevation	Mean temp.	Max. temp.	Min. temp.	Rainfall
Elevation	**1**	**−0.96**	**−0.95**	**−0.95**	**0.27**
Mean temp.		**1**	**0.99**	**0.99**	**−0.31**
Max. temp.			**1**	**0.95**	**−0.37**
Min. temp.				**1**	**−0.25**
Rainfall					**1**

Appendix 6

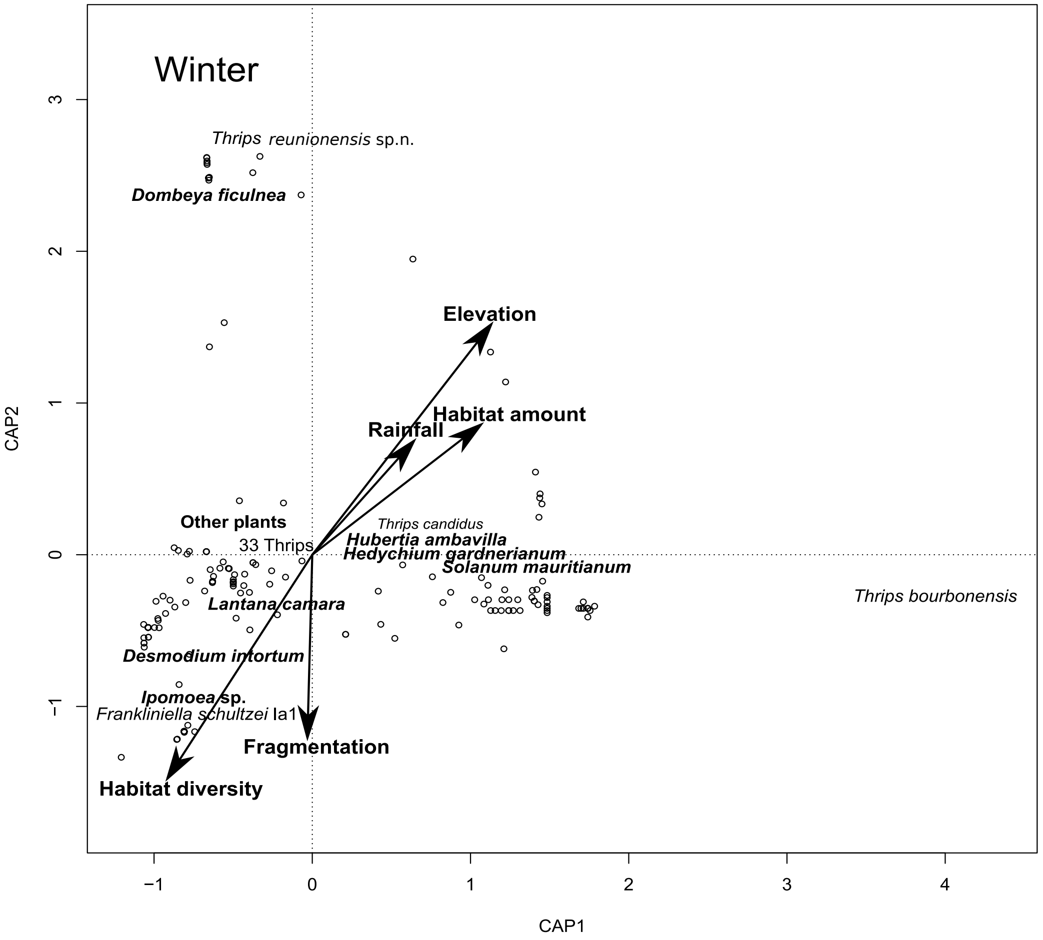
Family	Genus	Species	Status	Sampling number(*)
Malvaceae	*Abutilon*	*indicum*	Exotic	1(1)
Fabaceae	*Acacia*	*heterophylla*	Indigenous	1(1)
Fabaceae	*Acacia*	*meanrsii*	Exotic	2(1)
Fabaceae	*Acacia*	*farnesiana*	Exotic	1(0)
Asteraceae	*Ageratum*	*conyzoides*	Exotic	4(4)
Apocynaceae	*Allamanda*	*blanchetti*	Exotic	1(1)
Apocynaceae	*Allamanda*	*cathartica*	Exotic	1(1)
Amaranthaceae	*Amaranthus*	*spinosus*	Exotic	1(1)
Polygonaceae	*Antigonon*	*leptopus*	Exotic	2(1)
Rubiaceae	*Bertiera*	*rufa*	Indigenous	1(1)
Urticaceae	*Boehmeria*	*penduliflora*	Exotic	2(0)
Nyctaginaceae	*Bougainvillea*	sp.	Exotic	4(4)
Solanaceae	*Brugmansia*	*suaveolens*	Exotic	1(1)
Fabaceae	*Caesalpinia*	*pulcherrima*	Exotic	3(1)
Fabaceae	*Cajanus*	*cajan*	Exotic	2(2)
Myrtaceae	*Callistemon*	*speciosus*	Exotic	1(0)
Rubiaceae	*Chassalia*	*corallioides*	Indigenous	1(1)
Poaceae	*Chloris*	*barbata*	Exotic	1(1)
Cucurbitaceae	*Coccinia*	*grandis*	Exotic	1(1)
Asteraceae	*Conyza*	*sumatrensis*	Exotic	2(2)
Iridaceae	*Crocosmia*	*×crocosmiiflora*	Exotic	2(1)
Fabaceae	*Crotalaria*	*berteroana*	Exotic	1(1)
Poaceae	*Cynodon*	*dactylon*	Indigenous	1(1)
Orchidaceae	*Cynorkis*	*purpurascens*	Indigenous	2(1)
Cyperaceae	*Cyperus*	*polystachyos*	Exotic	1(1)
Solanaceae	*Datura*	sp.	Exotic	1(1)
Fabaceae	***Desmodium***	***intortum***	**Exotic**	**5(5)**
Plantaginaceae	*Digitalis*	*purpurea*	Exotic	3(1)
Sapindaceae	*Dodonaea*	*salicifolia*	Indigenous	2(0)
Sapindaceae	*Dodonaea*	*viscosa*	Indigenous	1(1)
Malvaceae	*Dombeya*	*punctata*	Indigenous	1(0)
Malvaceae	*Dombeya*	*elegans*	Indigenous	1(0)
Malvaceae	*Dombeya*	*ciliata*	Indigenous	1(0)
Malvaceae	***Dombeya***	***ficulnea***	**Indigenous**	**12(12)**
Verbenaceae	*Duranta*	*repens*	Exotic	1(0)
Poaceae	*Eleusine*	*aegyptica*	Exotic	1(1)
Ericaceae	*Erica*	*reunionensis*	Indigenous	2(1)
Asteraceae	*Erigeron*	sp.	Exotic	1(0)
Asteraceae	*Erigeron*	*karvinskianus*	Exotic	2(2)
Rosaceae	*Eriobotrya*	*japonica*	Exotic	1(1)
Euphorbiaceae	*Euphorbia*	*fulgens*	Exotic	1(1)
Moraceae	*Ficus*	*rubra*	Indigenous	1(1)
Escalloniaceae	*Forgesia*	*racemosa*	Indigenous	1(0)
Onagraceae	*Fuchsia*	*×exoniensis*	Exotic	1(0)
Onagraceae	*Fuchsia*	*boliviana*	Exotic	1(0)
Malvaceae	*Guazuma*	*ulmifolia*	Exotic	1(1)
Zingiberaceae	***Hedychium***	***gardnerianum***	**Exotic**	**15(10)**
Boraginaceae	*Heliotropium*	*indicum*	Exotic	2(1)
Campanulaceae	*Hippobroma*	*longiflora*	Exotic	1(1)
Lamiaceae	*Holmskioldia*	*sanguinea*	Exotic	1(1)
Asteraceae	***Hubertia***	***ambavilla***	**Indigenous**	**7(5)**
Hydrangeaceae	*Hydrangea*	*macrophylla*	Exotic	6(2)
Hypericaceae	*Hypericum*	*lanceolatum*	Indigenous	6(2)
Asteraceae	*Hypochaeris*	*radicata*	Exotic	1(0)
Balsaminaceae	*Impatiens*	*flaccida*	Exotic	1(1)
Convolvulaceae	*Ipomoea*	*carnea*	Exotic	1(0)
Convolvulaceae	***Ipomoea***	**sp.**	**Exotic**	**27(22)**
Verbenaceae	***Lantana***	***camara***	**Exotic**	**81(69)**
Fabaceae	*Leucaena*	*leucocephala*	Exotic	1(1)
Sapindaceae	*Licthi*	*sinensis*	Exotic	1(1)
Oleaceae	*Ligustrum*	*lucidum*	Exotic	3(3)
Lauraceae	*Litsea*	*glutinosa*	Exotic	1(0)
Malvaceae	*Malvastrum*	*coromandelianum*	Exotic	1(1)
Rutaceae	*Melicope*	*obtusifolia*	Indigenous	1(0)
Cucurbitaceae	*Momordica*	*charantia*	Exotic	2(1)
Asteraceae	*Montanoa*	*hibiscifolia*	Exotic	1(0)
Stilbaceae	*Nuxia*	*verticillata*	Indigenous	4(4)
Oxalidaceae	*Oxalis*	*tetraphylla*	Exotic	1(0)
Poaceae	*Panicum*	*maximum*	Exotic	9(3)
Asteraceae	*Parthenium*	*hysterophorus*	Exotic	1(1)
Poaceae	***Paspalum***	***dilatatum***	**Exotic**	**12(10)**
Poaceae	*Paspalum*	*urvillei*	Exotic	2(1)
Poaceae	*Paspalum*	*paniculum*	Exotic	1(1)
Poaceae	*Pennisetum*	*setaceum*	Exotic	1(0)
Poaceae	*Pennisetum*	*purpureum*	Exotic	3(2)

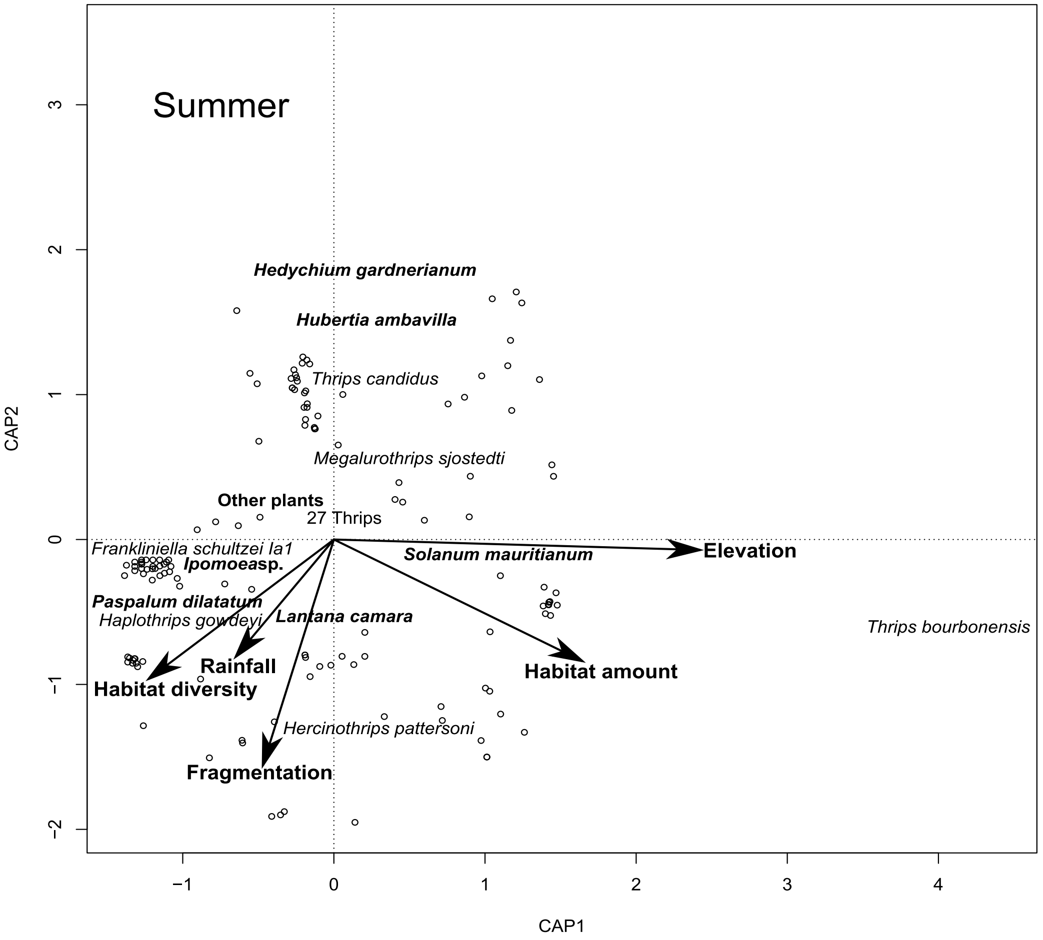
Asteraceae	*Pseudogynoxys*	*chenopodioides*	Exotic	1(0)
Asteraceae	*Psiadia*	*retusa*	Indigenous	2(2)
Anacardiaceae	*Rhus*	*longipes*	Exotic	3(3)
Euphorbiaceae	*Ricinus*	*communis*	Exotic	3(1)
Polygonaceae	*Rumex*	*abyssinicus*	Exotic	1(0)
Plantaginaceae	*Russelia*	*equisetiformis*	Exotic	1(1)
Poaceae	*Saccharum*	*officinarum*	Exotic	1(0)
Lamiaceae	*Salvia*	*coccinea*	Exotic	1(0)
Anacardiaceae	*Schinus*	*terebinthifolia*	Exotic	6(3)
Poaceae	*Setaria*	*pumila*	Exotic	1(1)
Solanaceae	***Solanum***	***mauritianum***	**Exotic**	**93(89)**
Solanaceae	*Solanum*	*torvum*	Exotic	2(1)
Asteraceae	*Sonchus*	*oleraceus*	Exotic	1(1)
Poaceae	*Sorghum*	*arundinaceum*	Exotic	2(1)
Myrtaceae	*Syzygium*	*jambos*	Exotic	1(0)
Asteraceae	*Tagetes*	*patula*	Exotic	2(2)
Apocynaceae	*Thevetia*	*peruviana*	Exotic	2(1)
Melastomataceae	*Tibouchina*	*grandifolia*	Exotic	4(3)
Melastomataceae	*Tibouchina*	*urvilleana*	Exotic	1(1)
Zygophyllaceae	*Tribulus*	*cistoides*	Exotic	1(1)
Tropaeolaceae	*Tropaeolum*	*majus*	Exotic	1(1)
Fabaceae	*Ulex*	*europaeus*	Exotic	3(3)
Unknown	Unkown	Unknown	Exotic	1(0)
Unknown	Unkown	Unknown	Exotic	1(1)
Unknown	Unkown	Unknown	Exotic	1(0)
Unknown	Unkown	Unknown	Exotic	1(1)
Unknown	Unkown	Unknown	Exotic	1(1)
Unknown	Unkown	Unknown	Exotic	1(1)
Verbenaceae	*Verbena*	sp.	Exotic	1(1)
Verbenaceae	*Verbena*	*bonariensis*	Exotic	3(3)
Cunoniaceae	*Weinmannia*	*trinctoria*	Indigenous	1(1)

Appendix 7

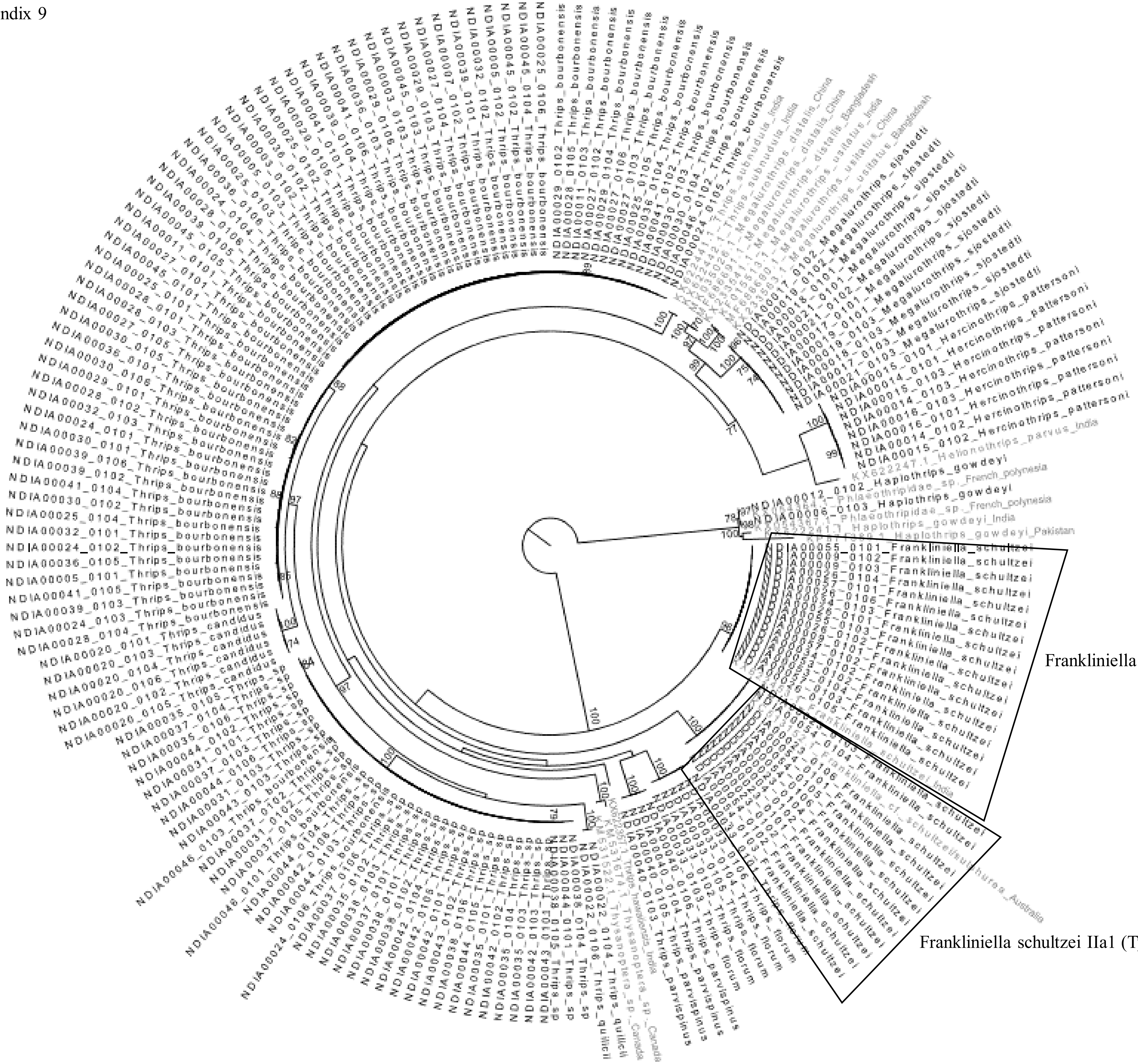
Scientific name	**Sub-order**	**Abundance**
Arorathrips mexicanus (Crawford, 1909)	Terebrantia	4
Ceratothripoides brunneus (Bagnall, 1918)	Terebrantia	7
Chaetanaphothrips orchidii (Moulton, 1907)	Terebrantia	2
Chirothrips sp.	Tubulifera	3
Dendrothripoides innoxius (Karny, 1914)	Terebrantia	3
Elaphothrips sp.	Tubulifera	1
Frankliniella insularis (Franklin, 1908)	Terebrantia	36
Frankliniella intonsa (Trybom, 1895)	Terebrantia	1
Frankliniella occidentalis (Pergande, 1895)	Terebrantia	29
Frankliniella schultzei Ia1 (Trybom, 1910, Tyagi et al., 2017)	Terebrantia	441
Frankliniella schultzei IIa1 (Trybom, 1910, Tyagi et al., 2017)	Terebrantia	77
Franklinothrips vespiformis (Crawford, 1909)	Terebrantia	10
Gigantothrips elegans (Zimmermann, 1900)	Tubulifera	1
Gynaikothrips ficorum (Marchal, 1908)	Tubulifera	2
Haplothrips articulosus (Bagnall, 1926)	Tubulifera	56
Haplothrips gowdeyi (Franklin, 1908)	Tubulifera	381
Haplothrips nigricornis (Bagnall, 1910)	Tubulifera	8
Haplothrips sp.	Tubulifera	5
Heliothrips haemorrhoidalis (Bouché, 1833)	Terebrantia	2
Hercinothrips pattersoni (Bagnall, 1919)	Terebrantia	198
Liothrips sp.	Tubulifera	11
Megalurothrips sjostedti (Trybom, 1910)	Terebrantia	199
Microcephalothrips abdominalis (Crawford, 1910)	Terebrantia	16
Neohydatothrips samayunkur (Kudo, 1995)	Terebrantia	6
Nesothrips sp.	Tubulifera	17
Scirtothrips aurantii (Faure, 1929)	Terebrantia	20
Scirtothrips sp.	Terebrantia	3
Scolothrips rhagebianus (Priesner, 1950)	Terebrantia	1
Thrips australis (Bagnall, 1915)	Terebrantia	1
Thrips bourbonensis (Bournier, 2000)	Terebrantia	1964
Thrips candidus (Bourniner and Bournier, 1988)	Terebrantia	114
Thrips florum (Schmutz, 1913)	Terebrantia	27
Thrips hawaiiensis (Morgan, 1913)	Terebrantia	11
Thrips palmi (Karny, 1925)	Terebrantia	31
Thrips parvispinus (Karny, 1922)	Terebrantia	70
Thrips quilicii (Bournier, 2000)	Terebrantia	150
Thrips reunionensis n. sp. (Goldarazena et al. 2020)	Terebrantia	329
Thrips sp.	Terebrantia	10
Thrips tabaci (Lindeman, 1889)	Terebrantia	14
Phlaeothripinea sp.	Tubulifera	1
Thripinae sp.	Terebrantia	17
Total species		**41**
Total abundances		**4279**

Appendix 8


Winter
Thrips reunionensis sp.n.
Dombeya ficulnea
Elevation
Rainfall
Habitat amount
Other plants
33 Thrips
Thrips candidus
Hubertia ambavilla
Hedychium gardnerianum
Solanum mauritianum
Thrips bourbonensis
Lantana camara
Desmodium intortum
Ipomoea sp.
Frankliniella schultzei Ia1
Fragmentation
Habitat diversity
CAP2
CAP1
3
2
1
0
−1
−1
0
1
2
3
4


Summer
Hedychium gardnerianum
Hubertia ambavilla
Thrips candidus
Megalurothrips sjostedti
Other plants
27 Thrips
Frankliniella schultzei Ia1
Ipomoeasp.
Solanum mauritianum
Elevation
Paspalum dilatatum
Haplothrips gowdeyi
Lantana camara
Thrips bourbonensis
Rainfall
Habitat diversity
Habitat amount
Hercinothrips pattersoni
Fragmentation
CAP2
CAP1
3
2
1
0
−1
−2
−1
0
1
2
3
4

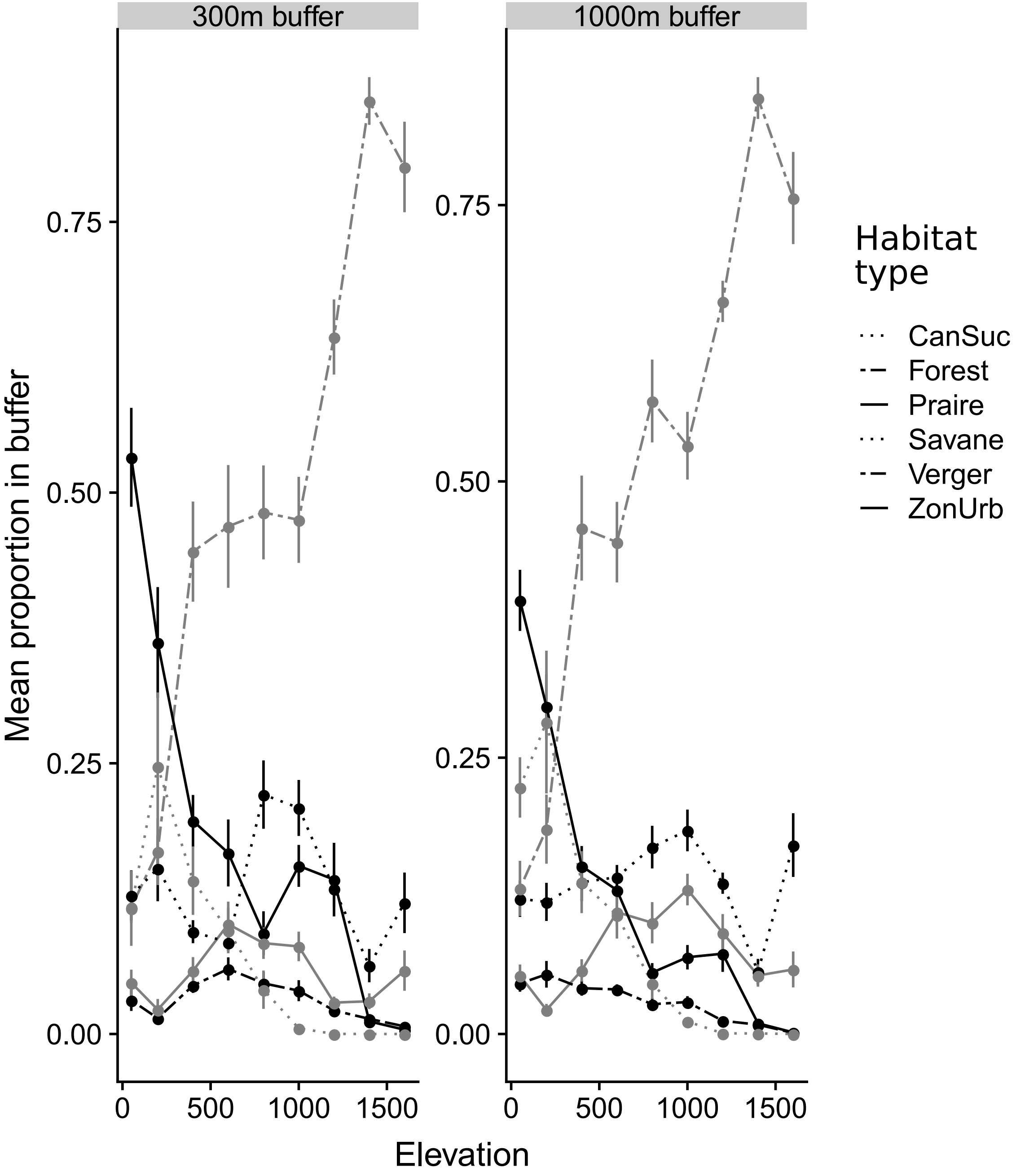
Appendix 9



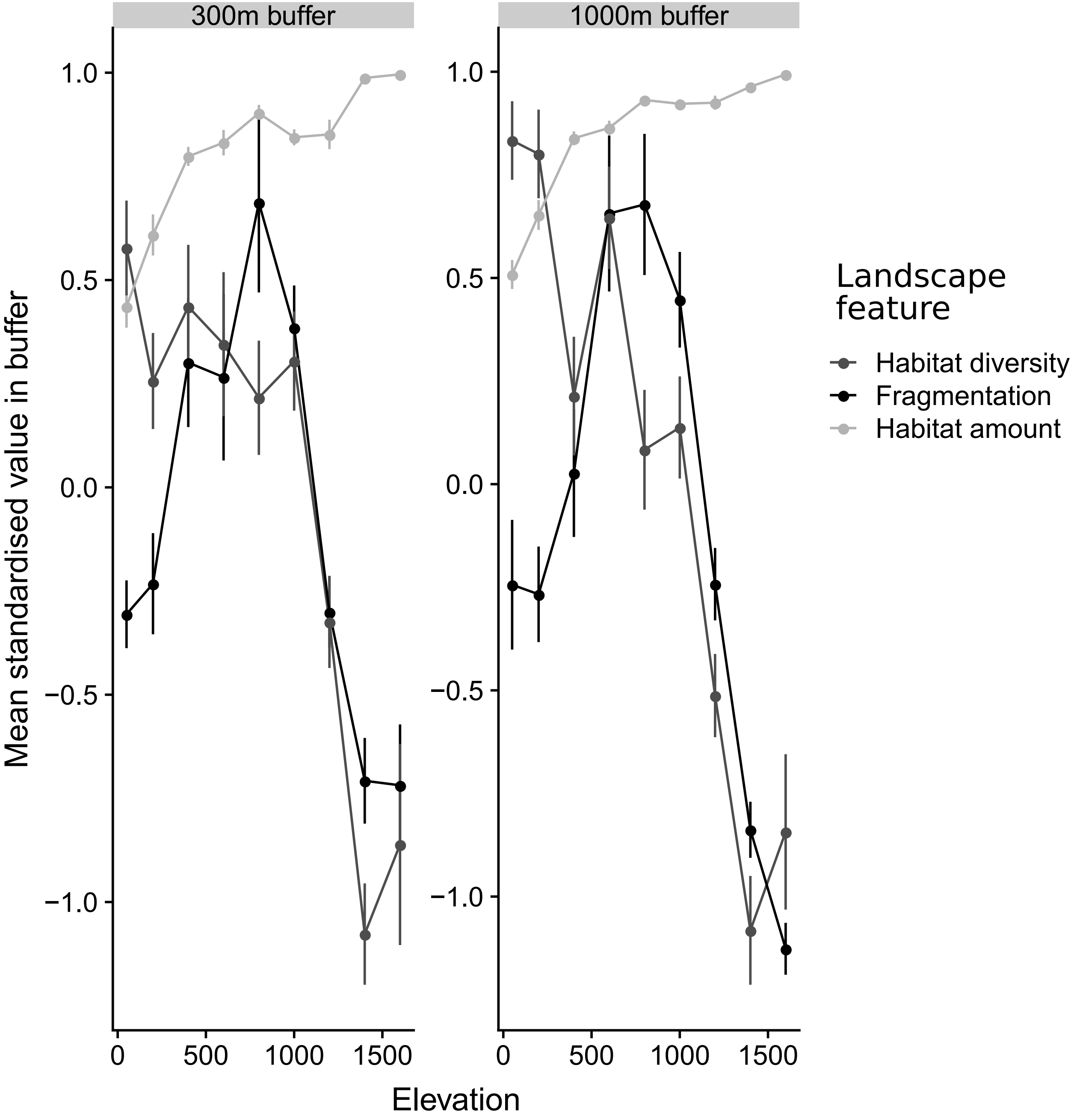
Frankliniella schultzei Ia1 (Tyagi et al. 2017)

Frankliniella schultzei IIa1 (Tyagi et al. 2017)

Appendix 10


300m buffer
1000m buffer
Mean proportion in buffer
0.00
0.25
0.50
0.75
0
500
1000
1500
Elevation
Habitat type
CanSuc
Forest
Praire
Savane
Verger
ZonUrb

Appendix 11


300m buffer
1000m buffer
Mean standardised value in buffer
1.0
0.5
0.0
−0.5
−1.0
0
500
1000
1500
Elevation
Landscape feature
Habitat diversity
Fragmentation
Habitat amount

Appendix 12

Multi-scale analysis

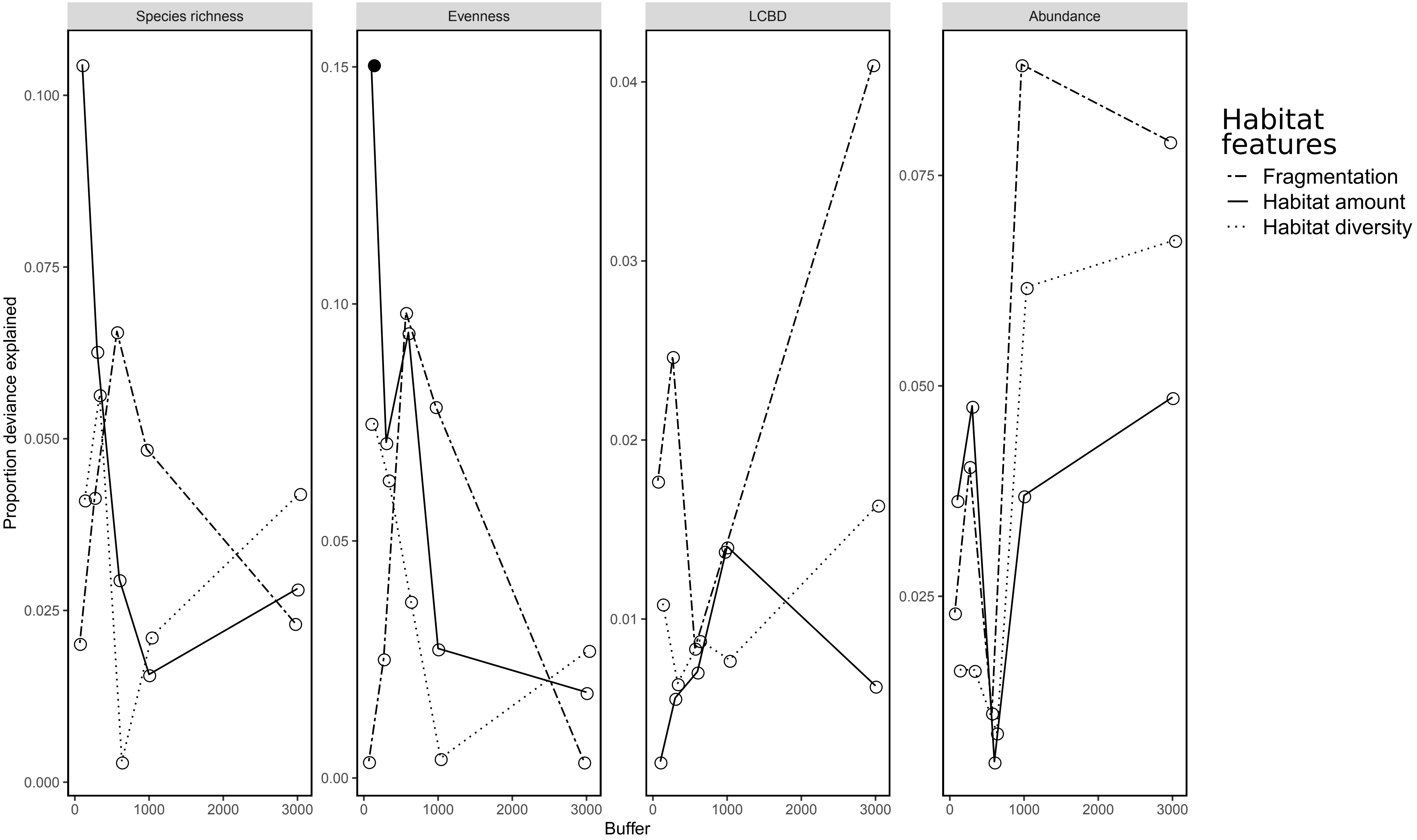
Methods

A multi-scale analysis was done by estimating the effect of landscape variables at different buffer sizes including circles of 100, 300, 600, 1000 and 3000 m of radius from the centre of each sampling site. These distances to the centre of the sampling site may represent short and long-distance dispersal of thrips. It is expected that the variance explained by the different landscape variables in statistical models is highest for the scale that best fits the home ranges or life dispersal of the species studied. The peak is thus the scale at which landscape variables operate at the maximum level. To perform this analysis, independent mixed effects models including all explanatory variables were built for each of the different response variables (i.e. abundance and insect diversity estimates). Independent models were also built using landscape data from each of the five different buffer sizes, leading to a total of 60 models (details on mixed effects models are provided below). To assess the variance explained by a given landscape variable in each model, the variance explained by full models was compared to the variance explained by simpler models in which the variable and its interactions were removed. In this analysis, the variance explained by the models was obtained with the function *r.squaredGLMM* from the package *MuMIn*, and significance of a given landscape variable in the different models was tested by comparing simplified and complex models. Within the same response and predictor variable, but along the different buffer sizes, p-values were corrected for multiple testing using the false discovery rate method.

Results

The deviance explained by the different landscape features varied greatly among landscape features and diversity metrics, and none of the buffer sizes used explained model variation greater than the others (Appendix S10). For species richness and the evenness (i.e. Simpson's diversity index) a non-consistent pattern was observed. For two out of the three landscape features studied, the variance explained in the LCBD models peaked at 3000m, whereas for insect abundance the largest deviance explained was obtained when landscape data was extracted from 1000m buffers. Based on this result, it was not possible to identify the buffer at which spatial effects were strongest and models were therefore built using landscape data from two different buffer sizes that may explain short and long-scale effects, 300m and 1000m, respectively.

Appendix 12



Species richness
Evenness
LCBD
Abundance
Proportion deviance explained
Buffer
0.000
0.025
0.050
0.075
0.100
0.00
0.05
0.10
0.15
0.01
0.02
0.03
0.04
0.025
0.050
0.075
0
1000
2000
3000
Habitat features
Fragmentation
Habitat amount
Habitat diversity

Appendix 13

100m BUFFER

	Richness			Evenness			LCBD			Abundance		
	Chisq	d.f.	p-val	Chisq	d.f.	p-val	Chisq	d.f.	p-val	Chisq	d.f.	p-val
Summer												
Plant	10.55	6	0.103	10.67	6	0.099	**40.48**	**6**	**<0.001**	**24.62**	**6**	**<0.001**
Elevation	0.15	1	0.701	0.01	1	0.917	**14.50**	**1**	**<0.001**	0.04	1	0.847
Precipitation	**5.78**	**1**	**0.016**	**4.47**	**1**	**0.034**	2.07	1	0.150	0.75	1	0.386
Habitat diversity	1.51	1	0.219	1.50	1	0.221	0.50	1	0.481	0.02	1	0.892
Fragmentation	1.73	1	0.189	1.93	1	0.164	0.44	1	0.507	0.20	1	0.655
Habitat amount	1.61	1	0.205	3.23	1	0.073	0.31	1	0.578	0.02	1	0.887
Hab. div X Fragm.	0.03	1	0.870	0.22	1	0.635	0.03	1	0.859	1.80	1	0.180
Hab. div X Hab. am.	2.31	1	0.129	**4.38**	**1**	**0.036**	0.35	1	0.557	0.24	1	0.623
Fragm. X Hab. am.	0.23	1	0.633	0.27	1	0.606	0.21	1	0.647	0.67	1	0.415
Total variance explained			0.330			0.268			0.396			0.208
Winter												
Plant	6.32	7	0.503	**22.54**	**7**	**0.002**	**91.93**	**7**	**<0.001**	**80.17**	**7**	**<0.001**
Elevation	3.37	1	0.066	**8.50**	**1**	**0.004**	1.56	1	0.211	1.41	1	0.235
Precipitation	0.70	1	0.403	1.87	1	0.171	0.49	1	0.482	0.39	1	0.534
Habitat diversity	0.11	1	0.738	1.28	1	0.257	3.71	1	0.054	0.06	1	0.809
Fragmentation	0.02	1	0.889	0.17	1	0.683	2.41	1	0.120	0.10	1	0.754
Habitat amount	1.00	1	0.316	1.14	1	0.285	3.20	1	0.074	0.03	1	0.860
Hab. div X Fragm.	0.18	1	0.669	0.34	1	0.561	0.73	1	0.394	0.01	1	0.908
Hab. div X Hab. am.	0.13	1	0.722	0.97	1	0.326	0.23	1	0.630	0.48	1	0.490
Fragm. X Hab. am.	0.04	1	0.842	1.20	1	0.274	1.33	1	0.248	0.50	1	0.479
Total variance explained			0.102			0.261			0.447			0.493

600m BUFFER

	Richness			Evenness			LCBD			Abundance		
	Chisq	d.f.	p-val	Chisq	d.f.	p-val	Chisq	d.f.	p-val	Chisq	d.f.	p-val
Summer												
Plant	10.92	6	0.091	11.44	6	0.076	**41.59**	**6**	**<0.001**	**28.64**	**6**	**<0.001**
Elevation	0.03	1	0.863	<0.01	1	0.996	**11.96**	**1**	**0.001**	0.14	1	0.706
Precipitation	**8.72**	**1**	**0.003**	**5.64**	**1**	**0.018**	1.51	1	0.220	0.27	1	0.603
Habitat diversity	2.02	1	0.155	2.78	1	0.095	<0.01	1	0.955	1.55	1	0.213
Fragmentation	0.04	1	0.848	1.14	1	0.285	0.79	1	0.373	1.05	1	0.306
Habitat amount	1.04	1	0.307	2.97	1	0.085	0.09	1	0.770	<0.01	1	0.982
Hab. div X Fragm.	1.63	1	0.202	1.80	1	0.180	0.22	1	0.641	0.02	1	0.891
Hab. div X Hab. am.	0.47	1	0.494	0.56	1	0.456	0.14	1	0.706	0.53	1	0.466
Fragm. X Hab. am.	1.06	1	0.303	1.83	1	0.177	0.14	1	0.708	0.64	1	0.425
Total variance explained			0.327			0.288			0.403			0.201
Winter												
Plant	6.32	7	0.503	**22.84**	**7**	**0.002**	88.00	7	0.000	**80.03**	**7**	**<0.001**
Elevation	3.16	1	0.075	**6.81**	**1**	**0.009**	1.88	1	0.171	0.61	1	0.435
Precipitation	1.09	1	0.296	1.11	1	0.292	0.07	1	0.798	0.17	1	0.684
Habitat diversity	0.24	1	0.625	0.73	1	0.392	1.27	1	0.259	0.04	1	0.839
Fragmentation	0.12	1	0.733	1.14	1	0.285	2.85	1	0.091	2.06	1	0.152
Habitat amount	0.51	1	0.477	0.10	1	0.752	**4.24**	**1**	**0.039**	0.13	1	0.714
Hab. div X Fragm.	0.09	1	0.769	<0.01	1	0.994	0.01	1	0.931	0.12	1	0.734
Hab. div X Hab. am.	0.20	1	0.652	0.11	1	0.743	0.07	1	0.796	0.28	1	0.597
Fragm. X Hab. am.	1.00	1	0.316	0.26	1	0.610	0.01	1	0.938	0.95	1	0.331
Total variance explained			0.086			0.278			0.436			0.485

3000m BUFFER

	Richness			Evenness			LCBD			Abundance		
	Chisq	d.f.	p-val	Chisq	d.f.	p-val	Chisq	d.f.	p-val	Chisq	d.f.	p-val
Summer												
Plant	11.29	6	0.080	12.01	6	0.062	**42.00**	**6**	**<0.001**	**28.62**	**6**	**<0.001**
Elevation	0.96	1	0.327	0.04	1	0.835	**10.14**	**1**	**0.001**	0.31	1	0.580
Precipitation	**6.71**	**1**	**0.010**	**4.53**	**1**	**0.033**	2.56	1	0.109	0.18	1	0.670
Habitat diversity	0.10	1	0.753	0.27	1	0.604	0.01	1	0.944	0.49	1	0.483
Fragmentation	0.08	1	0.782	0.68	1	0.409	1.18	1	0.277	<0.01	1	0.955
Habitat amount	1.38	1	0.240	0.78	1	0.377	0.01	1	0.921	<0.01	1	0.993
Hab. div X Fragm.	0.02	1	0.898	0.14	1	0.707	0.03	1	0.868	0.07	1	0.788
Hab. div X Hab. am.	**8.18**	**1**	**0.004**	**5.05**	**1**	**0.025**	0.05	1	0.827	0.05	1	0.831
Fragm. X Hab. am.	<0.01	1	0.961	0.04	1	0.846	0.67	1	0.413	0.49	1	0.485
Total variance explained			0.336			0.263			0.430			0.189
Winter												
Plant	5.63	7	0.584	**22.81**	**7**	**0.002**	**87.57**	**7**	**<0.001**	**79.15**	**7**	**<0.001**
Elevation	2.11	1	0.146	**5.75**	**1**	**0.016**	0.62	1	0.432	0.04	1	0.842
Precipitation	<0.01	1	0.994	0.86	1	0.353	<0.01	1	0.975	2.20	1	0.138
Habitat diversity	0.84	1	0.361	0.72	1	0.395	1.50	1	0.221	**4.42**	**1**	**0.035**
Fragmentation	2.66	1	0.103	0.06	1	0.811	1.62	1	0.204	**6.78**	**1**	**0.009**
Habitat amount	0.29	1	0.593	0.08	1	0.778	1.05	1	0.306	0.19	1	0.664
Hab. div X Fragm.	0.31	1	0.575	0.13	1	0.719	0.56	1	0.454	0.28	1	0.596
Hab. div X Hab. am.	0.27	1	0.607	0.47	1	0.495	<0.01	1	0.975	1.30	1	0.255
Fragm. X Hab. am.	0.03	1	0.862	<0.01	1	0.965	0.53	1	0.466	1.72	1	0.190
Total variance explained			0.105			0.282			0.437			0.507

CHAPTER FOUR

Microbial symbionts of herbivorous species across the insect tree

Enric Frago[a,b], **Sharon E. Zytynska**[c], **Nina E. Fatouros**[d]
[a]CIRAD, CBGP, Montpellier, France
[b]CBGP, CIRAD, INRA, IRD, Montpellier SupAgro, University Montpellier, Montpellier, France
[c]Institute of Integrative Biology, Department of Evolution, Ecology and Behaviour, University of Liverpool, Liverpool, United Kingdom
[d]Biosystematics Group, Wageningen University and Research, Wageningen, The Netherlands

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Abstract

Microbes play crucial roles in the biology of herbivorous insects, and the last decade has provided exciting new evidence for a prominent role of microbial symbiosis in detoxification of plant toxins, manipulation of plant defences and defence against natural enemies. We provide an order by order update of symbioses across herbivorous insects, particularly focusing on recent published evidence, and on how symbionts interact

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with the defensive system of the plant. While the hemimetabolous Hemiptera order largely relies on obligatory microbial symbioses, we did not find such a close relationship between symbionts and hosts in the other three orders Orthoptera, Phasmatodea and Thysanoptera. These three orders mostly harbour transient gut symbionts and/or rely on laterally transferred genes from microbes. Despite the radical changes and harsh conditions during metamorphosis, numerous holometabolous species transmit symbionts vertically and show close associations with both intra- and extracellular symbionts. The last section of this book chapter discusses the role that symbionts will play in future scenarios of global warming, but also their implications for the transmission of plant viruses and modern agriculture.

1. Introduction

The natural world is full of examples of intricate symbiotic interactions between species like the coevolution of orchid bees and orchids, or ants and acacia trees. Many interactions are not seen by the naked eye but can have dramatic impacts for the individuals involved. Insects have evolved obligate (required) and facultative (helpful, but not required) symbiotic associations with bacteria, fungi and protozoans. Symbiotic interactions in insect herbivores have provided key knowledge into the ecology and evolution of animal symbioses (Douglas, 2011; McCutcheon et al., 2019), exemplifying how such interactions can influence animal diet and defence against natural enemies. Such unseen interactions are the focus of this chapter.

Technological advances, and molecular biology in particular, have made the study of insect-microbe interactions more open and accessible across the world. This has resulted in a quick increase in the number of research papers describing different systems, yet there is still a bias towards model systems that have been studied for longer. Unsurprisingly, this has also led to a rise in the number of reviews and forward-looking perspectives on this topic that consider broader groups (Douglas, 2015; Flórez et al., 2015; Hammer and Bowers, 2015; Moran et al., 2019), yet many are taxon-focused (Biedermann and Vega, 2020; Dicke et al., 2020; Duplouy and Hornett, 2018; Kaltenpoth and Engl, 2014; Mason et al., 2019a; McLean et al., 2016; Paniagua-Voirol et al., 2018; Sudakaran et al., 2017). We present a systematic overview of symbionts within the different orders of herbivorous insects (those that feed on living tissue of vascular plants). In each section we present an overview of symbiosis in the group and highlight relevant recent papers together with any previous reviews that go into more detailed specifics for that group. Our aim for this chapter is to present a broad overview of insect-symbiont-plant interactions, and an appreciation of the potential impacts these can have in the future.

Insects have evolved herbivory several times, and particularly within eight orders of winged insects classified together within the subclass Pterygota (McKenna et al., 2019; Wiens et al., 2015). Herbivorous species-rich clades, i.e. Hemiptera (stink, true and shield bugs, leaf and tree hoppers, psyllids, whiteflies, aphids, and cicadas), Lepidoptera (moths and butterflies) and Coleoptera (beetles) make up the vast majority of the approximately 450,000 described herbivore species (Stork, 2018). Feeding on plants is challenging, often because of high levels of toxins or low levels of nutrients, and due to this many insect herbivores have evolved symbiotic relationships with microbes that have enabled them to feed on this low-quality food source (Hammer and Bowers, 2015). In some cases, herbivorous insects have taken up beneficial genes from the microbes (horizontal gene transfer) leaving the microbe itself obsolete to the insect (McKenna et al., 2019; Shelomi et al., 2016; Wybouw et al., 2016). About half of all described insect species are herbivores. So the question arises whether this great success of insect herbivory is largely due to vertically-transmitted beneficial symbionts and/or ancient lateral gene transfers from bacteria, viruses and/or fungi. Alternatively, opportunistic microbes, co-opted from the plants' phyllosphere or the soil, could further benefit these herbivores. For example, there are Lepidopteran species whose larvae have been shown to largely consist of transient gut bacterial communities rather than resident symbionts (Hammer et al., 2019b; Mason et al., 2019a; Moran et al., 2019; Paniagua-Voirol et al., 2018).

Plants have a complex defensive system. These defences are considered constitutive when they are always present, or induced when they are triggered upon insect attack. Constitutive defences often come from the secondary metabolism of the plant and include a whole suite of compounds that exert toxic effects on insects (Schoonhoven et al., 2005). These defences, however, can be costly and most plants have also evolved a defensive layer that is only induced once the plant perceives the attack by the insect, and which is similar to the immune system of animals. Induced defences can be either direct or indirect. Direct ones are aimed at killing, poisoning or repelling herbivores, whereas indirect ones enhance the effectiveness of the attacker's natural enemies (Dicke and Baldwin, 2010; Hilker and Fatouros, 2015; Pieterse et al., 2013). Plant volatiles are important in mediating induced indirect defences as they are capable of making often inconspicuous herbivores more easily located by predators and parasitic wasps and flies (Turlings and Erb, 2018). One way that plants fine-tune their antiherbivore defences is via phytohormones, which are induced differently depending, among other factors, on the specific stressor that attacks them (Stam et al., 2013). While the jasmonic acid (JA) pathway is mainly induced through attack by leaf-chewing insects and some phloem feeders, the salicylic acid (SA) pathway mainly acts upon induction by phloem feeders and plant pathogens. Both pathways 'crosstalk' so that upregulation of one pathway can downregulate the other, especially under multiple attack, i.e. JA-SA crosstalk (Erb et al., 2012; Lazebnik et al., 2014). Some insects that trigger induced plant defences upon feeding have evolved counter defensive strategies that down-regulate these responses. These strategies can be achieved using the intrinsic metabolic repertoire of the insect (Erb and Reymond, 2019), or by co-opting this function from a microbial symbiont. During the last few years, numerous studies have indicated symbiont roles in modulating plant hormones and antiherbivore defences, and, in particular, altering crosstalk between JA and SA pathways. These discoveries motivated several review papers, some of them very recent (Casteel and Hansen, 2014; Engel and Moran, 2013; Frago et al., 2012; Giron et al., 2013, 2017; Hammer and Bowers, 2015; Mason et al., 2019a; Oliver and Martinez, 2014; Shikano et al., 2017; Sugio et al., 2015).

1.1 Scope of this review

This review will focus on microbial symbionts of herbivorous species, and particularly their effects on host plant use. There have been some recent reviews on this topic, and this is why we decided to perform an order by order revision, focusing on recent publications: we hereby focus on four hemimetabolous and four holometabolous insect orders altogether containing most herbivorous insect species. The last decade has seen an important amount of research on defensive insect symbioses. This topic has also been extensively reviewed in recent years (Flórez et al., 2015; Kaltenpoth and Engl, 2014; Monticelli et al., 2019; Oliver et al., 2010; Zytynska and Meyer, 2019), and will not be the main focus of this book chapter. However, the most well studied examples will be discussed, particularly when defensive symbioses may help understanding host plant use by herbivores. Manipulation of plant defences might imply symbiont colonization of the plant and replication in it, and this is commonly the case of plant pathogens that are vectored by insects. Although in these systems the insect has often been considered a passive vector, on many occasions the pathogen becomes a mutualist because the insect obtains a benefit from feeding on a diseased plant (Frago et al., 2012). This type of symbiosis has been largely documented in phloem feeding Hemiptera, particularly between aphids and thrips that vector viruses, but also between psyllids and bacteria. Since these types of interactions have also been recently reviewed by Eigenbrode et al. (2018), we will discuss here cases in which symbionts help their hosts manipulate plant physiology without systemically colonizing the plant. A last section is also included where the implications of symbionts in insect herbivores in a changing world are discussed.

2. Hemimetabola

The development of hemimetabolous insects is characterized by a series of moults, leading to an adult stage with wings. From the 18 hemi metabolous insect orders, four of them largely contain herbivorous species, namely Orthoptera (grasshoppers and crickets), Phasmatodea (walking sticks), Thysanoptera (thrips) and the largest one, Hemiptera (including, among others, aphids, shield bugs, tree and plant hoppers and whiteflies) (Fig. 1). Hemimetabolous insects are more likely to harbour stable symbiotic associations than holometabolous ones because their body plan is more stable over development (Hammer and Moran, 2019). Many species are associated with obligate symbionts that allow them to exploit otherwise unusable feeding niches, as seen with hemipterans in the suborder Sternorrhyncha that are specialized on plant phloem sap (see below) (Sudakaran et al., 2017; Wernegreen, 2004). Yet, other hemimetabolous lineages such as the Phasmatodea seem to have evolved a lifestyle largely independent of microbial symbionts (Shelomi et al., 2013, 2016).

2.1 Orthoptera

The few studies dissecting the microbial community of grasshoppers and crickets mainly focus on edible species sold for human consumption (Stoops et al., 2016) or pest species (Idowu et al., 2009). Orthoptera are chewing insects that macerate foliar tissue using strong mandibles. Evidence shows resident gut microbial communities in Orthoptera are rare, and that the microbiome is mostly acquired from the environment (Mason et al., 2019a). For example, guts of first instar variegated grasshoppers, *Zonocerus variegatus*, hatching from eggs were found to be sterile (Ademolu and Idowu, 2011). Further, Dillon & Charnley (2002) found that the gut microbiota of desert locusts, *Schistocerca gregaria* (Fig. 2A) was dominated by Enterobacteriaceae found in

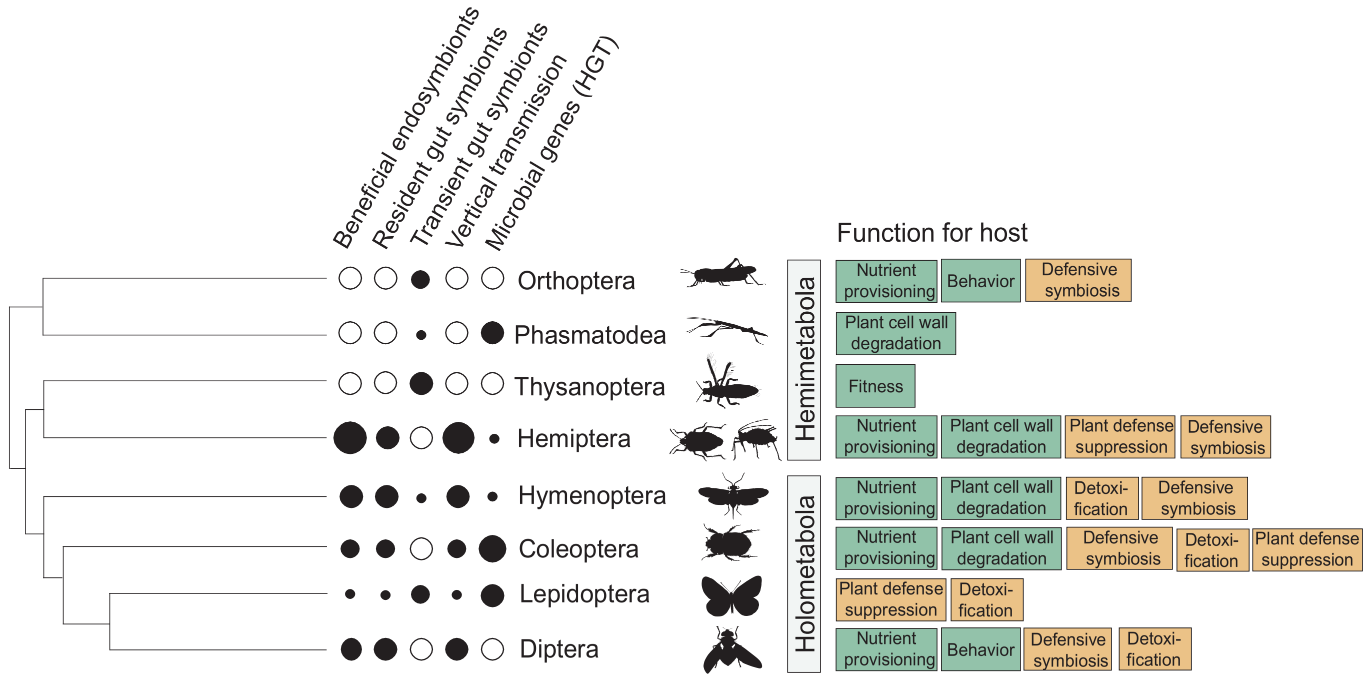

Fig. 1 Different types of symbionts, transmission routes and functions for hosts across herbivorous insect orders using the phylogenetic tree from Misof et al. (2014). Herbivorous insects are distributed across four hemimetabolous and four holometabolous orders in total consisting of about 450,000 species (Wiens et al., 2015). Traits can be very common (large black circle), less common (small black circle), or absent or not known/studied (white circle). Green boxes represent traits that are essential for the host, while orange ones represent benefits against natural enemies or plants defences.

Fig. 2 Examples of herbivorous insects and interactions with microbial symbionts: (A) through gut microbial metabolism, the desert locust *Schistocerca gregaria* produces phenolics used as protection against pathogens and/or components of the locusts' aggregation pheromone, (B) phasmids like *Clonopsis gallica* acquire plant cell wall degrading enzymes from bacteria by horizontal gene transfer, (C) western flower thrips *Frankliniella occidentalis* are associated with γ-proteobacteria like *Erwinia* and *Pantoea*, (D) aphids host up to nine common endosymbionts, and the symbiont *Regiella insecticola* protects the green peach aphid *Myzus persicae* from parasitoids, (E) whiteflies like *Bemisia tabaci* are reported to host seven facultative symbionts namely *Rickettsia*, *Wolbachia*, *Hamiltonella*, *Arsenophonus*, *Cardinium*, *Fritschea*, and *Hemipteriphilus*, (F) psyllids like *Psylla pyri* are associated with *Carsonella ruddii* symbionts, (G) the bean bug *Riptortus pedestris* (Vengolis, https://commons.wikimedia.org/wiki) harbours *Burkholderia* gut symbionts that confers them with insecticide resistance, (H) termites host microbiomes that are predominantly composed of resident, beneficial microbes, (I) fungus growing leaf-cutter ants keep vertically transmitted *Streptomyces* bacteria on their cuticle that produce antibiotics to suppress fungus garden-parasites, (J) solitary bees show a great diversity

(Continued)

the environment, suggesting they are acquired after hatching. Despite the transient nature of these associations, the relationship between some of the acquired bacteria and locusts seems to be mutualistic. Secondary plant chemicals like phenolics, are degraded by microbial metabolism in the locusts' gut and the products used as protection against pathogens and/or components of the locusts' aggregation pheromone (Dillon and Charnley, 2002; Dillon et al., 2000). Previous digestion of plant material in the locusts' gut, however, appeared to be a prerequisite for the production of phenolics by the gut bacteria. Microbial transformation of plant secondary metabolites may be widespread in this order, as other Orthoptera species were also shown to have antimicrobial phenolics in their gut fluid (Dillon and Charnley, 2002).

2.2 Phasmatodea

To break down ingested foliar tissue or to facilitate plant penetration, herbivorous insects depend on plant cell wall degrading enzymes (PCWDEs) like cellulases and pectinases, that are often produced by symbiotic bacteria. However, recent studies suggest that phasmids (stick insects) do not depend on gut symbionts for plant digestion, but potentially on transient bacteria

Fig. 2—Cont'd and variability in their gut microbiome depending on the environment and the way microbes are transmitted, (K) tenthredinid sawfly larvae show a low apparent diversity of gut bacteria mainly attributed to the host plant, (L) the tortoise beetle *Cassida rubiginosa* acquired an extracellular bacterium, *Stammera*, that took over pectinase activities to digest plant cell wall components, (M) some bark and ambrosia beetles like the European Shot-hole Borer *Anisandrus dispar* are fungal farmers and transport their symbiotic fungi in mycangia, (N) oral secretions of Colorado potato beetle *Leptinotarsa decemlineata*, contain gut bacteria deposited into wounds that suppress plant defences, (O) extracellular *Burkholderia gladioli* bacteria were shown to protect eggs of Lagriinae beetles (Tenebrionidae) from pathogenic microbes, (P) the microbiome of caterpillars of the cabbage moth *Mamestra brassicae* is modulated by bacteria and fungi present in the soil and host plant, (Q) eggs and hatching caterpillars of the cabbage white butterfly *Pieris brassicae* contain low amounts of bacteria which do not affect egg-mediated priming of plant defences, (R) the microbiome of different wild mushroom-feeding *Drosophila* species are very similar, probably reflecting similarities in the ecological niche exploited, (S) tephritids like the Cherry fruit fly *Rhagoletis cerasi* carry in their guts yeasts and bacteria that are transferred to the fruit by females during oviposition. *Part A: credits: Antoine Foucart; Part B: credits: Nina E. Fatouros; Part C: credits: Tibor Bukovinszky; Part D: credits: Nina E. Fatouros; Part E: credits: Tibor Bukovinszky; Part F: credits: Tibor Bukovinszky; Part H: credits: Nina E. Fatouros; Part I: credits: Nina E. Fatouros; Part J: credits: Jitte Groothuis; Part K: credits: Nina E. Fatouros; Part L: credits: Nina E. Fatouros; Part M: credits: Peter H.W. Biedermann; Part N: credits: Nina E. Fatouros; Part O: credits: Martin Kaltenpoth; Part P: credits: Tibor Bukovinszky; Part Q: credits: Tibor Bukovinszky; Part R: credits: Jitte Groothuis; Part S: credits: Tibor Bukovinszky.*

obtained from the diet or environment (Shelomi et al., 2015). Similar to caterpillars and Orthoptera, the gut of phasmids is a straight and narrow tube lacking chambers that could harbour obligate symbionts. In addition, egg-smearing, coprophagy or other known modes of transmission of resident symbionts that are found in other groups like cockroaches (Blattodea) and termites (Isoptera) are absent. This evidence suggests that vertical transmission of stable symbiont communities is unlikely in this group (Salem et al., 2015). Next generation sequencing and microscopy of two phasmid species revealed that the only heritable symbionts were *Spiroplasma*, which can manipulate the reproduction of parthenogenetic species (Shelomi et al., 2013).

The lack of PCWDE-producing heritable symbionts is likely due to ancient horizontal transfer of microbial genes. A major clade of phasmids (the Euphasmatodea that includes all lineages but Timematodea) produces pectinases endogenously without the need for a microbe (Fig. 2B). This evolutionary novelty was likely acquired from bacteria by horizontal gene transfer because the most similar homologues of these genes were found in γ-proteobacteria (Shelomi et al., 2016). Horizontal gene transfer of pectinase genes has also been shown for beetles, butterflies and moths (see below), but the phasmatodean genes are not related to the latter two (Shelomi et al., 2016). Whether the pectinase genes identified in Euphasmatodea were acquired from stable symbiotic associations or from transient microbes occurring in ancestral stick insect species is a question that still needs to be clarified.

2.3 Thysanoptera

The role of microbial symbionts has been investigated only in a few species of thrips, mainly pest species. Some herbivorous thrips seem to harbour gut bacteria, consisting largely of Enterobacteriaceae. In a recent review, Schausberger (2018) presents an overview of the endosymbiotic and gut/saliva bacteria from the thrips family Thripidae, which contains most pest species. Of particular interest are two of the most widespread pest species, the western flower thrips, *Frankliniella occidentalis* (Fig. 2C) and the onion thrips, *Thrips tabaci*, both associated with γ-proteobacteria, including *Erwinia* and *Pantoea* or *Pantoea*-like bacteria. Empirical studies have shown *Erwinia* to positively affect thrips fitness, yet the transmission of these gut bacteria is likely not vertical and they are probably acquired from the plant surface (De Vries et al., 2004). *Pantoea* bacteria are also likely to be transmitted horizontally among thrips via the plant. This bacterium causes plant diseases like centre rot in onion, and is vectored by the onion-infesting thrips *F. fusca*. The bacteria are transmitted to the plant and other thrips through faeces and persist in thrips through several life stages (Dutta et al., 2016). It is not known, however, if this bacterium confers any fitness advantage to its host, so its condition of insect mutualist remains to be demonstrated. A more recent study on *T. tabaci* identified a gut bacterial community mainly consisting of Proteobacteria (Gawande et al., 2019). In this study, the diversity and structure of the bacterial community depended on the environment and habitat where thrips lived. Based on this evidence, thrips seem to primarily acquire microbes from their environment (Dickey et al., 2014), and evidence for beneficial endosymbionts and vertically transmitted resident gut bacteria is thus scarce in this group. This contrasts with the closely-related order Hemiptera, probably reflecting the difference in feeding mode (i.e. cell-content feeding vs phloem feeding).

2.4 Hemiptera

Phloem-feeding Hemiptera, and aphids in particular, have long been used as model systems to study nutritional symbioses. Plant phloem is of poor nutritional quality and feeding exclusively on this substrate is challenging because many essential amino-acids that are usually needed for animal development are lacking. To complement this poor diet, many phloem-feeding Hemiptera have engaged in obligatory associations with nutritional bacteria (Douglas, 1998). Acquisition of these symbionts was at the core of the diversification of many groups, including *Buchnera aphidicola* in aphids, *Portiera aleyrodidarum* in whiteflies and *Carsonella ruddii* in psyllids (Dolling, 1991; Moran et al., 2008) (Fig. 2D–F). There are many reviews on the evolution and mechanistic functioning of obligatory symbioses in hemipterans (Douglas, 1998, 2015; Moran et al., 2005; Sudakaran et al., 2017) so we will cover this topic here in less detail, and focus on the facultative associations that are not required for survival. The role of hemipteran facultative endosymbionts in host plant use, adaptation to abiotic conditions and interactions with natural enemies has been studied for more than a decade, particularly in well-established model species like aphids and whiteflies (Brownlie and Johnson, 2009; Frago et al., 2012; Guo et al., 2017; Kanakala and Ghanim, 2019; Moran et al., 2008; Oliver et al., 2010, 2014; Vorburger, 2018; Zytynska and Meyer, 2019; Zytynska and Weisser, 2016). Studies on these types of symbioses are mostly limited to bacteria, with some fungi examples like that of the brown planthopper, *Nilaparvata lugens*

(Chen et al., 1981). Little effort has been made to identify the importance of other microbial groups, including protozoa, which may be reasonably suspected to play important roles as well.

2.4.1 Aphids

Aphid endosymbionts are predominantly vertically transmitted from mother to offspring during clonal reproduction, with some reported cases of transmission failure in the field depending on the symbiont species, other hosted symbionts, and host genotype (Rock et al., 2018). Horizontal transfer of symbionts among aphids can also occur during sexual reproduction, via the host plant, by parasitic wasps when ovipositing eggs into the aphids, or even through infected honeydew (reviewed in Chrostek et al., 2017 and Zytynska and Weisser, 2016). Thus, on average, only 40–60% of aphids in a population are infected by even the most prevalent facultative symbiont.

Aphid facultative symbionts have been particularly well studied in the pea aphid, *Acyrthosiphon pisum*, and have been found to provide a variety of services to their hosts including protection against parasitic wasps, predators, fungal pathogens and heat shock (reviewed by Guo et al., 2017; Oliver et al., 2010, 2014). Whether facultative symbionts play a role in host-plant specialization in this species is a question that has long been debated. This taxon is considered as a complex of species because it has a continuum of populations that are specialized on various plant species in the Fabaceae family, and strong associations between particular species of symbionts and host plants are observed (Oliver et al., 2010; Peccoud et al., 2009; Peccoud and Simon, 2010; Tsuchida et al., 2002; Via et al., 2000). Tsuchida et al. (2004) provided the first experimental evidence of a nutritional role of facultative symbionts in *A. pisum*. This study showed that the facultative γ-proteobacterium, called pea aphid U-type symbiont (PAUS; now known as *Regiella insecticola*) had a positive effect on host fitness when feeding on white clover, *Trifolium repens*, and this effect could be transferred to another aphid *Megoura crassicauda* by artificially transferring the symbiont. This result was not recovered, however, in a later study by Leonardo (2004) revealing that the effect of the symbiont depended on the specific combination of aphid and symbiont genotypes. A more recent study that tracked the evolutionary history of the pea aphid taxon using phylogenetic techniques revealed that acquisition of particular symbiont species coincided with the colonization of novel host plants (Henry et al., 2013). Facultative symbionts thus played a role in the colonization of new host plants, but whether this was due to nutritional provisioning is a question that cannot be answered through phylogenetic inference. The main reason is that, in *A. pisum* some facultative symbionts are known to increase their hosts' resistance against natural enemies and environmental stressors (Flórez et al., 2015; Oliver et al., 2010, 2014), which are factors that may modulate colonization of new host plants. As suggested by McLean et al. (2016), for example, *A. pisum* aphids collected on clover often carry the symbiont *Regiella insecticola*, which is also known to confer resistance against the specialist aphid fungal pathogen *Pandora*. Is it possible that acquisition of this symbiont allowed aphids to colonize clover, but that this was selected because pressure by the natural enemy was stronger on this plant? This idea has been explored in a few studies, often with mixed results (Hrček et al., 2016; Sochard et al., 2019) as not all symbiont genotypes protect likewise, and there are strong interactions between the protection conferred by the symbiont and the intrinsic resistance that symbiont-free aphids may also possess.

Two closely related symbiont species *Hamiltonella defensa* and *Regiella insecticola* stand out as defensive symbionts that protect their hosts against parasitic wasps and fungal pathogens, respectively (Flórez et al., 2015; Oliver et al., 2010, 2014). Facultative symbionts are not usually considered as nutritional, but in some species they have been found to replace, or complement the nutritional services of the primary symbiont *Buchnera*, thus becoming obligatory (Sudakaran et al., 2017). In *Cinara cedri*, *Serratia symbiotica* was the first example of a facultative symbiont taking over the synthesis of essential nutrients (tryptophan and riboflavin) from *Buchnera* (Lamelas et al., 2011). A more recent study also found that many *Cinara* aphids also carry *Erwinia* symbionts, which live within their own bacteriocytes near to those of *Buchnera* (Manzano-Marin et al., 2020). These authors show that this symbiont has vitamin-synthesizing genes that compensate for deficiencies in the *Cinara-Buchnera* interaction, and even provide a new function potentially synthesizing biotin and thiamine for the aphid. Moreover, in one aphid lineage a subset of genes was found to have been transferred to a different symbiont (*H. defensa*) suggesting that lateral gene transfers could drive the establishment and dynamics of multi-symbiont communities.

2.4.2 Whiteflies

Whiteflies and psyllids are sister lineages with a common ancestor that established a long-term association with a bacterium, which diversified and provided each group their obligate symbiont (Santos-Garcia et al., 2018). Whiteflies are reported to host seven facultative symbionts around the world namely *Rickettsia*, *Wolbachia*, *Hamiltonella*, *Arsenophonus*, *Cardinium*, *Fritschea*, and *Hemipteriphilus* (Kanakala and Ghanim, 2019). The whitefly *Bemisia tabaci* forms a cryptic species complex, with sibling species now considered distinct at 4% genetic divergence. However, across these species genetically-similar facultative symbionts (i.e. same genetic cluster) were identified—with symbionts from the same genetic cluster hosted by highly-divergent host species (Kanakala and Ghanim, 2019). Relative to aphids, whitefly facultative symbionts seem thus to be more readily transferred from one individual to another by horizontal transmission (Ahmed et al., 2013; Qi et al., 2019). The transfer by parasitoid wasps has been shown to occur rather frequently, but only for specific symbiont species. For example, a study in China identified five symbiont species in the whitefly (*Hamiltonella*, *Arsenophonus*, *Cardinium*, *Rickettsia* and *Wolbachia*) and only the latter two were found in a common parasitic wasp of the whitefly (Qi et al., 2019). In this study symbiont horizontal transmission was confirmed because of shared symbiont haplotypes. Similar to *Cinara* aphids, a co-obligate relationship between whitefly primary and various facultative symbionts has been shown to provide nutritional benefits to the host. For example, the whitefly facultative symbiont *Hamiltonella*, which is often found sharing bacteriocytes with the obligate *Portiera*, is thought to produce several B vitamins (Rao et al., 2015). While research on symbiosis in whiteflies and other non-aphid hemipterans is a little behind that on aphids, we find that there are some similarities (e.g. types of symbiont, or action of effect) but also many differences (e.g. horizontal transmission rates) between the systems.

2.4.3 Stinkbugs, true bugs and shield bugs

The hemipteran suborder Heteroptera includes herbivorous families like Pentatomidae, Acanthosomatidae, Alydidae and Scutelleridae (i.e. stinkbugs, true bugs and shield bugs). In these groups, nutritional bacteria are not endosymbionts that live within host tissues, but microbes hosted in specialized gut structures. Removal of these bacteria using antibiotics often leads to dramatic increases in insect mortality, which suggests that in most species these microbes are obligatory (Zytynska et al., 2019). In some species these symbionts have also been found to confer other important services. For example, the bean bug (*Riptortus pedestris*) (Fig. 2G) harbours *Burkholderia* gut symbionts that the nymphs acquire every generation from the soil. The bacteria were shown to confer insecticide resistance to the pest insects, quickly established within a single generation and potentially transferred horizontally to other pests (Kikuchi et al., 2012).

2.4.4 Symbionts modulate induced plant defences in Hemiptera

As far as we are aware, endosymbiont manipulation of plant defences was first reported in the whitefly *B. tabaci*. When infected with the symbiont *Hamiltonella*, the insect downregulated tomato defences for its own benefit (Su et al., 2015). The authors of this report also showed that the effect was lost in plant mutants that were deficient in salicylic acid dependent responses, which revealed that this strategy is based on the manipulation of the balance between opposing phytohormonal routes (i.e. the aforementioned crosstalk). This result evidenced a mechanism of symbiont manipulation of plant defences that has been found in other insect groups (see below), and which consists in cheating the plant by inducing salicylic acid defences that are usually triggered against pathogens, leading to a down-regulation of insect-specific defences that depend on jasmonic acid. In the grain aphid *Sitobion miscanthi*, the symbiont *Hamiltonella* has also been found to down-regulate wheat defences, although not by altering the balance between the two phytohormones mentioned (Li et al., 2019). In this study, the symbiont down-regulated all defences that the authors measured, including jasmonic and salicylic routes, but also polyphenol oxidase (PPO) and peroxidase (POD). Although plant defences in grasses are less well understood than in tomato, this study presents an alternative strategy in which the symbiont does not target a specific phytohormonal route, but probably a more global defensive mechanism found downstream of the phytohormonal routes mentioned.

Plant defences do not only act against herbivores by directly affecting their fitness, but also indirectly by attracting herbivores' natural enemies, for example via the emission of attractive plant volatiles. As mentioned before, in the pea aphid *A. pisum*, the symbiont *H. defensa* is known to protect aphids against parasitic wasps and predators (Flórez et al., 2015) by preventing the development of the wasp's larva or by reducing the fitness of predators. In a recent study, this defensive effect has been found to extend to parasitic wasp recruitment (Frago et al., 2017). *Hamiltonella defensa* reduced plant volatile emissions, rendering plants less attractive to the wasp *Aphidius ervi*, and ultimately reducing attacks on young aphids. Based on this and previous studies, *H. defensa* thus stands out as a symbiont capable of manipulating plant defences upon aphid and whitefly feeding. Surprisingly, however, the study by Frago et al. (2017) revealed that the symbionts *Regiella*, *Spiroplasma*, *Serratia* and *Rickettsiella* also manipulated the emission of plant volatiles and plant attraction to the wasp. This result suggests that many symbionts, and not only *H. defensa*, may play a role in manipulation of plant physiology in aphids.

The mechanism by which hemipteran endosymbionts challenge induced plant defences is so far little understood. Three potential (and non-exclusive) explanations are possible: (1) injection of salivary effectors of bacterial origin into the plant, (2) effectors of bacterial origin delivered to the plant via honeydew, or (3) behavioural changes in the insect. The first mechanism is the most likely occurring in the whitefly example discussed above (Su et al., 2015) because the authors succeeded in emulating manipulation of plant defences by artificially injecting the saliva of the insect into the plant. Although in this example the symbiont was the facultative *H. defensa*, this mechanism can potentially involve obligatory symbionts, as *Buchnera* proteins have been found in the saliva of the aphids *A. pisum* and *Megoura viciae* (Vandermoten et al., 2014). Plant sap is rich in sugars but poor in proteins, and for this reason phloem feeders excrete the excess sugar and produce honeydew. Working with the pea aphid *A. pisum*, Schwartzberg and Tumlinson (2014) demonstrated for the first time that experimental honeydew deposition suppressed jasmonic acid in plants while increasing levels of salicylic acid. Although the honeydew also contained the latter phytohormone, the levels in plants were larger, so that the plant played a role in such an increase. This study did not look for hidden microbial players in mediating plant responses, even if many proteins of symbiont origin are found in the honeydew of this same aphid species (Sabri et al., 2013). Although it is possible that symbionts (or proteins of symbiont origin) in the honeydew manipulate plant responses for the benefit of the insect hosts, most research pinpoints honeydew as the Achilles heel in these associations. For instance, in the aphid *Macrosiphum euphorbiae*, GroEL proteins that originate from the obligatory symbiont *Buchnera* are responsible for triggering plant defences (Chaudhary et al., 2014). In the brown planthopper, *Nilaparvata lugens*, which is one of the most important pests of rice, honeydew-associated microbes elicited plant defences and even the release of plant volatiles (Wari et al., 2019). More precisely, the authors ‘cured’ insects from bacterial associates using antibiotics and demonstrated that bacteria amplified antiherbivore defences, specifically phytoalexins. By culturing microbes obtained from honeydew, the authors also identified the species *Acinetobacter soli* and *Serratia marcescens* as potential suspects that the plant uses as clues of insect attack. Symbionts in aphid honeydew can also betray their hosts by increasing attraction of their natural enemies as found in *A. pisum* aphids whose gut resident *Staphylococcus sciuri* directly produces volatiles that attract aphid natural enemies (Leroy et al., 2011).

The last potential mechanism whereby symbionts may allow insects to manipulate plant defences is by altering their host’s behaviour. Aphids need to penetrate plant tissues with their mouthparts, and before starting to suck plant sap they often perform several punctures known as probing behaviours. While doing so, the plant suffers from mechanical damage and the insect releases salivary effectors into plant tissues, which are two important elements for the plant to recognize the attacker, but also for the insect to avoid detection. Symbionts are increasingly recognized as having an influence on animal behaviour (Rohrscheib and Brownlie, 2013; Su et al., 2013b), and a recent study demonstrated that the facultative symbiont *H. defensa* alters probing behaviour in the aphid *Rhopalosipum padi* (Leybourne et al., 2020). The change in feeding behaviour due to symbiont carrying led to a less efficient nutritional intake and reduced insect fitness. It is possible, however, that in other hemipteran species, or host plants, these behavioural alterations weaken plant induced defences.

3. Holometabola

Holometabola is the most species-rich animal lineage comprising about 850,000 described species (i.e. 50% of all animal species) (Beutel and Pohl, 2006). Development through a complete metamorphosis is seen as its key success (Beutel et al., 2011). Yet, the radical change in form and function from larval to adult life stage is a constraint for the acquisition of vertically transmitted symbionts (Hammer and Moran, 2019). A complete metamorphosis implies that larval organs break down and may not be reformed during the adult stage: symbionts in the gut or bacteriomes need to reallocate and recolonize newly-formed structures. Moreover, symbionts need to withstand severe conditions during metamorphosis in the pupal phase. This has led to the evolution of alternative transmission routes that bypass metamorphosis. These alternatives mostly include externally transferred symbionts via inoculation of the substrate (in *Drosophila*, for instance), or social transmission (known from ants and bees, and in hemimetabolous insects such as cockroaches and termites) (Salem et al., 2015) (Fig. 2H). Unlike in hemimetabolous insects, microbiota of Holometabola can change dramatically between larvae and adults as they often exploit different feeding niches. This diversion in feeding niches is especially apparent in Lepidoptera and herbivorous Hymenoptera (sawflies and gall wasps) because larvae are folivorous or xylophagous while adults feed on nectar or pollen (Hammer and Moran, 2019). Despite these difficulties, many holometabolous insects possess vertically transmitted symbionts that have found ways to counter the constraints imposed by metamorphosis (Fig. 1).

3.1 Hymenoptera

About 15% of the ca. 150,000 hymenopteran species are phytophagous. This estimate includes bees that feed on nectar and pollen, ants that feed on plant exudates, fungus-growing ants (including leaf-cutter ants) (Fig. 2I), plant-galling wasps, and the paraphyletic group of basal hymenopteran lineages (previously named Symphyta) that includes sawflies, horntails, and wood wasps. Many hymenopterans have established intimate nutritional symbioses with fungi. As reviewed by Biedermann and Vega (2020), herbivorous hymenopteran groups having symbiotic associations with fungi include fungus-growing ants that harvest plants upon which fungi are cultivated and then fed upon, wood wasps that inoculate fungi into the xylem to feed their solitary larvae, and stingless bees that feed their larvae with a culture of fungi growing in nectar. These fungi mostly facilitate digestion of low-quality diets like wood or leaves, but they also degrade and detoxify plant defensive compounds. Regarding bacterial mutualists, the honey bee gut microbiome has become a model of host-microbe interactions and has been intensely discussed in previous reviews (Douglas, 2019; Engel et al., 2016; Zheng et al., 2018). Distinctive gut bacterial lineages are vertically transmitted through social contacts likely facilitating host-symbiont coevolution (Engel and Moran, 2013; Moran et al., 2019). Yet, this social-bee model is not representative for the vast majority of bees that are solitary (>90% of the >17,500 species) (Fig. 2J). Relative to social bees, solitary ones show a greater diversity and variability in their gut microbiome, which strongly depends on the environment and the way microbes are transmitted among individual insects (Voulgari-Kokota et al., 2019). A recent study has revealed that the microbiome of megachilid bees, for instance, depends on the flowers they visit (McFrederick et al., 2017), and Kim et al. (2019) found that pollinating Hymenoptera can even act as agents of horizontal transmission of plant symbionts. In this latter study, a *Streptomyces* strain moves from the rhizosphere into strawberry roots up to their flowers and is then transferred by pollinating honey bees to other flowers. Like in fungus-growing ants (see below), the *Streptomyces* strain studied protected the bees but also the plants from pathogens. Symbionts in the remaining herbivorous hymenopteran groups, mainly sawflies, ants and gall wasps have been largely studied too, and in our review, we detail results from recent studies.

Many ant species have acquired vertically-transmitted bacterial symbionts along their evolutionary history (Moreau, 2020). Most herbivorous ants are considered as canopy foragers that feed on plant exudates, insect honeydew, pollen and vertebrate waste, and they obtain little nitrogen even when they complement their diet via predation. In *Cephalotes*, *Dolichoderus* and *Camponotus* ants, genomic evidence suggests that bacteria located in the gut may perform useful nitrogen-metabolic services for their hosts (Bisch et al., 2018; Gil et al., 2003; Hu et al., 2018). Due to symbiont enrichment in herbivorous ants, and relatedness of some gut bacteria to nitrogen-fixing rhizobia that are mutualistic with leguminous plants, some authors have speculated that endosymbionts have facilitated the origins and maintenance of the 'herbivorous' lifestyle across this insect family (Kaltenpoth and Flórez, 2020; Russell et al., 2009; Stoll et al., 2007). Nitrogen fixation was originally posited as a mutualistic service, but in vivo demonstration of this activity by internally harboured ant symbionts has proven elusive, and shotgun (meta) genomic sequencing has similarly failed to identify nitrogen fixation genes in abundant endosymbionts. Such efforts have, however, implicated symbionts in mutualistic nitrogen recycling, with studies in both the *Cephalotes* and *Camponotus* systems combining in vivo experiments with genomics to support such roles (Feldhaar et al., 2007; Gil et al., 2003; Hu et al., 2018).

Beyond these taxa, fungus-growing leaf-cutting ants (subtribe Attina, genus *Atta* and *Acromyrmex*) have established intimate associations with the fungus they cultivate on harvested plant material (reviewed in Moreau, 2020). In addition to these associations, however, leaf-cutting ants have established symbiosis with bacteria. Although the gut microbiome is quite simple in attine ants, these species keep vertically-transmitted Actinobacteria (e.g. *Streptomyces*) on their cuticle that produce antibiotics to suppress fungus garden-parasites, an important service to ensure that only the right fungi grows on harvested plant material (Currie et al., 1999). These cuticular actinobacterial biofilms are vertically transmitted by many fungus-growing ant genera, which makes these species exceptional in keeping distinct microbiomes externally and internally. Notably, the composition of the gut microbiomes appears to be affected by the presence/absence of these other symbionts on the cuticle: gut microbiomes of ant species that carry cuticular Actinobacteria tend to be more similar than those without, especially in the ant lineages arising later in the history of this taxon (Sapountzis et al., 2019).

Besides interactions with cuticular symbionts, gut microbial symbionts can affect the social dynamics between leaf-cutter ants by changing the cuticular chemicals that ants use as recognition cues. In the leaf-cutting ant *Acromyrmex echinatior* antibiotic treatment led to a more aggressive behaviour against nestmates, which in turn correlated with a decrease in the abundance of two antifungal compounds, that are produced against fungus garden-parasites (Teseo et al., 2019). These compounds are produced by exocrine metapleural glands that are unique to ants and which also produce secretions with antibiotic properties that modulate Actinobacteria communities present on the ant cuticle (Poulsen et al., 2003). After antibiotic treatment, when ants were fed with faecal droplets the gut bacterial community was partly restored together with normal behaviour (Teseo et al., 2019).

Sawflies are a group of herbivorous Hymenoptera, comprising some species that are serious pests of wheat. A first comprehensive microbiota screening of six sawfly species representing four different Symphyta families (Agridae, Diprionidae, Pamphiliidae and Tenthredinidae) (Fig. 2K) revealed a low apparent diversity of gut bacteria. The bacteria found were mainly α- or γ-proteobacteria that were mainly attributed to the host plant (Graham et al., 2008). For example, *Rhanella* sp. found in half of the screened species may have been acquired from the host plant as they were isolated from foliage in other studies (e.g. Hashidoko et al., 2002). A more recent study revealed that these sawflies are colonized by a novel *Spiroplasma* species, detected in both adults and larvae that is likely to be either transmitted vertically or horizontally by larval feeding on the inner wheat stem (Yeoman et al., 2019). This symbiont carries several genes encoding for carbohydrate-metabolism as well as biosynthetic pathways of essential B-vitamins. Furthermore, *Spiroplasma* genes encode for cardiolipin synthase and chitinase, both potentially involved in insect defence, which would add sawflies as another insect group using *Spiroplasma* as a defensive symbiont (Ballinger and Perlman, 2019).

The hymenopteran lineage Cynipoidea comprises both plant and insect parasites. Within this lineage, the Cynipidae (about 1400 spp.) family is entirely specialized in forming galls mainly on oak trees and rose bushes (Ronquist et al., 2015). Gall wasps induce plants to modify their host physiology and develop complex gall structures that often resemble novel plant organs. Wasp larvae feed within the protective gall. A transcriptional analysis of ovaries and venom glands of two gall wasp species revealed that unlike many hymenopteran parasitoid species, gall wasps seem to be deprived of viral genes or virus particles in the venom glands, which could aid at reprograming plant cells for gall development (Cambier et al., 2019). However, similar to insect herbivores in other orders, it is likely that cellulase genes expressed in the venom glands and/or ovaries are of bacterial origin. The acquisition of such horizontally transferred genes might have been an important adaptation in the evolution of Cynipidae to become plant parasites.

Yet, a phylogenomic approach on the whole lineage is needed to further elucidate whether cellulase genes originate from horizontal gene transfer events as was shown for the Phasmatodea discussed above (Shelomi et al., 2016).

Defensive symbioses have been identified in herbivorous Hymenoptera, particularly against pathogens that threaten fungal gardens in leaf-cutter ants (as mentioned above) or bee larvae, but also against eukaryotic parasites (reviewed in Flórez et al., 2015 and Kaltenpoth and Engl, 2014). In the bumblebee *Bombus terrestis*, for instance, gut symbionts reduced infection rates by the trypanosomatid parasite *Crithidia bombi* (Koch and Schmid-Hempel, 2011).

3.2 Coleoptera

The enormous diversity of beetles is especially attributed to the adaptive radiation of specialized herbivorous beetles feeding on angiosperms. A recent phylogenetic study revealed that specialization mainly in leaf and seed mining, and stem and wood boring in the highly diverse Phytophaga lineage (longhorn beetles, leaf beetles, and weevils; >125,000 described species) was enabled by plant cell wall-degrading enzymes (PCWDEs) obtained from bacteria and fungi via horizontal gene transfer events (Kirsch et al., 2014; McKenna et al., 2019). The origin of genes associated with PCWDEs were found to be phylogenetically and temporally linked to significant increases in diversification rates some 200 million years ago. These enzymes obtained via horizontal gene transfer are therefore proposed as a key innovation that facilitated the evolution of specialized plant-feeding niches in herbivorous beetles, which account for almost half of all described beetle species (Coleoptera >400,000 species). Despite the importance of horizontal gene transfer for the success of this group, stable associations with microbes are also common in phytophagous beetles. This suggests an ongoing process of loss and replacement of genes related to cell-wall degradation, but also of microbes capable of performing this function (Kirsch et al., 2014; Salem et al., 2017).

Co-speciation between bacterial symbionts and leaf beetles (Chrysomelidae) has been shown for several species belonging to different subfamilies (Fukumori et al., 2017; Kölsch and Synefiaridou, 2012; Salem et al., 2017). In *Sitophilus* grain weevils, for example, intracellular symbionts provide essential amino acids (Vigneron et al., 2014). A more recent example has revealed that the tortoise leaf beetle *Cassida rubiginosa* (Fig. 2L) acquired an obligate symbiont that took over pectinase activities to digest plant cell wall components (Salem et al., 2017). This extracellular bacterium, *Stammera*, has a reduced genome, a common characteristic of endosymbionts in stable symbiotic associations (Moran et al., 2008). In this example, the bacteria are localized in sac-like structures connected to the foregut as well as in the females' reproductive tract, both evidence of stable associations. They are vertically transferred through the egg, which is topped with a cap-like structure enclosing spheres densely populated with *Stammera*. Such complex structures guarantee that the symbiont is encapsulated throughout the hosts' development and are more typical for partnerships where symbionts are transferred intracellularly during oogenesis (Salem et al., 2017). Even if horizontally acquired PCWDEs are widespread in beetles (McKenna et al., 2019), co-opting symbionts that produce these same molecules may be more common than currently thought particularly when means of vertical transmission and morphological adaptations to accommodate the microbes in the intestine have evolved (Mason et al., 2019a). However, how often a similar type of symbiosis such as with *Stammera* exists in other beetle lineages remains to be investigated.

Gut microbes have also been found to provide their hosts with essential amino acids, and this seems particularly common in species that feed on nutrient-limited food sources like wood (Biedermann and Vega, 2020). The Asian longhorned beetle, *Anoplophora glabripennis*, for instance, uses gut bacteria and fungi to obtain these organic compounds (Ayayee et al., 2016). Microbial co-option may not only be common to perform universal enzymatic reactions like pectinase activities, but also to digest specific plant secondary metabolites. For example, one of the most devastating pests of coffee, the coffee berry borer, *Hypothenemus hampei*, carries a *Pseudomonas* bacterium in the gut that degrades caffeine and allows insect survival on coffee fruits (Ceja-Navarro et al., 2015). Plant secondary metabolites can even lead to the convergence of gut microbial communities, particularly in well-defended host plants. This has been demonstrated in Cycads, which are a group of plants that have evolved tight interactions with the insects that pollinate them, as they interact through larval feeding on the plant as well as through pollination services. Cycads contain carcinogenic and neurotoxic compounds in their tissues and it is thought that insect development on them is facilitated by bacterial symbionts. Salzman et al. (2018) compared the microbiomes of three beetles and two butterflies that thrive on Cycads, and found that they share at least two bacteria that are absent in non-cycad feeding close relatives.

One of the coleopteran groups where symbiosis has been more extensively studied includes bark and ambrosia beetles (reviewed in Biedermann and Vega, 2020 and Hulcr and Stelinski, 2017) (Fig. 2M), probably because some species are devastating forest pests. Bark and ambrosia beetles have evolved tight associations with fungi. In this group, fungi are usually vertically transmitted and carried between plants in specialized structures in the beetles' elytra, and the fungi have even lost their capacity to reproduce sexually. Both adults and larvae of the beetle feed on fungal gardens, and the fungus can also physically block resin ducts, which can act as a plant defence against herbivores. The use of symbiotic fungi has also evolved in other beetle families including lizard beetles, ship-timber beetles and leaf-rolling weevils (reviewed in Biedermann and Vega, 2020). Some bark beetles like pine engravers and *Dendroctonus* spp. (both comprising severe pests of coniferous trees) also contain bacteria that decrease defensive monoterpenes produced by their host plants (Howe et al., 2018). As found in the cycad example mentioned above (Salzman et al., 2018), beetles seem to have convergent microbiomes strongly influenced by the host plants' defensive chemistry (Howe et al., 2018; Mason et al., 2019a).

Many beetles use microbes to defend themselves, particularly against pathogens as found in the bark beetle *Dendroctonus frontalis* and in leaf-rolling weevils in the genus *Euops* (reviewed in Biedermann and Vega, 2020 and Flórez et al., 2015). In Lagriinae beetles (Fig. 2O), extracellular bacteria were shown to protect eggs from pathogenic microbes (Kaltenpoth and Flórez, 2020). Symbiotic *Burkholderia gladioli* are present in the females' accessory reproductive glands and are smeared onto the eggs during oviposition. The bacteria then enter the egg and colonize the embryo. The symbionts produce an antifungal polyketide that protects the eggs from fungi present in the soil environment. Conceivably these symbionts were originally acquired from plants as they can cause disease in the beetles' host, and they can also be transmitted horizontally among beetles via the plant (Flórez et al., 2017). Besides lagriine beetles, multiple other insect taxa rely on the symbiosis with *Burkholderia* bacteria, which have been found to provide defensive and nutritional services, and also detoxification of insecticides (reviewed in Kaltenpoth and Engl, 2014). As suggested by these authors, it is likely that insects often acquire symbionts from the environment because these bacteria are widespread in the soil and on plants.

3.2.1 Symbionts modulate induced plant defences in Coleoptera

Bark and ambrosia beetles farm mutualistic fungi to feed both larvae and adults. These species attack conifers, which prevent insect colonization using resins enriched with toxic compounds like terpenes. At least in some conifer species, resin production is an induced defence as its production is increased after wounding (e.g. Lombardero et al., 2000). Beetle fungal symbionts can impair the efficiency of these defences by actively degrading the toxic compounds (e.g. Boone et al., 2013; Hammerbacher et al., 2013). In the pine weevil, *Hylobius abietis*, although diterpene acids had no toxic effect against the beetle, gut bacteria were found to be able to degrade these compounds and to provide the resulting, catabolized molecules as a nutritional food source for the insect (Berasategui et al., 2017). Beetle symbionts have also been found to manipulate plant defences downstream of the production of toxic compounds via altering defence-related genes and phytohormones. The Western corn rootworm, *Diabrotica virgifera*, is one of the most important pests of maize. A transcriptomic study found that beetles carrying the endosymbiont *Wolbachia* suppressed the induction of defence-related genes in the plant (Barr et al., 2010). This finding, however, was later contradicted by Robert et al. (2013) who showed that the symbiont did not alter the induction of specific markers of defence genes, or the emission of defensive plant volatiles. Bacterial symbionts not only colonize guts but find their way to salivary glands, enabling their occurrence in oral secretions. As also found in whiteflies and lepidopterans, oral secretions of two chrysomelids, the Colorado potato beetle (*Leptinotarsa decemlineata*) (Fig. 2N) and false potato beetle (*L. juncta*) are deposited into wounds during feeding to downregulate plant defences with a concomitant increase in insect performance (Chung et al., 2013, 2017; Wang et al., 2016). Based on a sequencing analysis of beetle regurgitates, similar roles have been suggested in the soybean pest *Epilachna varivestis* (Gedling et al., 2018). A detailed revision of these types of strategies can be found in Mason et al. (2019a). Symbionts in oral secretions benefit their hosts because they manipulate crosstalk between phytohormonal routes. While they still trigger the expression of salicylic acid-dependent genes (usually ineffective against insect attackers), they downregulate jasmonic acid-dependent responses that are specific against insect attackers. The first evidence of this strategy in beetles identified the symbionts belonging to the genera *Stenotrophomonas*, *Pseudomonas* and *Enterobacter* as responsible for this effect (Chung et al., 2013). In species using this strategy, the bacterial community in oral secretions may influence plant specialization because the microbiome of oral secretions depends on the host plant, and also because manipulation of plant defences depends on both the host plant and the insect species (Chung et al., 2017; Wang et al., 2016). Whether suppression of plant defences by symbiotic bacteria in oral secretions is widespread in chewing insects, and whether this mechanism helps them to conquer new host plants is a question that needs to be further investigated.

3.3 Lepidoptera

Although Lepidoptera is one of the most species-rich orders with almost exclusively folivorous feeding caterpillars, the role of microbial symbionts as nutritional partners remains ambiguous (Duplouy and Hornett, 2018; Paniagua-Voirol et al., 2018). Many recent studies provide evidence that caterpillars collected from the wild lack a resident gut microbiome, so that their guts are mostly colonized by transient and often diet-associated species (Hammer et al., 2017; Jones et al., 2019; Phalnikar et al., 2019; Staudacher et al., 2016; Szenteczki et al., 2019; Whitaker et al., 2016). As found in Coleoptera, evidence accumulates that ancient horizontal gene transfer events from microbes may have played a major role in the evolution of herbivory in Lepidoptera (Sun et al., 2013; Wybouw et al., 2014, 2016). The caterpillars' gut commonly harbours very low numbers of microbes, which is probably due to its harsh conditions of fast food passage and high pH levels, especially in the midgut (Engel and Moran, 2013; Paniagua-Voirol et al., 2018). Moreover, specialized tissues or structures that contain microbes, as are commonly found in other groups like Coleoptera or Hemiptera, have so far not been found in Lepidoptera. Gut microbial symbiosis is therefore unlikely to contribute to the diversification and evolution of specialization on Angiosperm families in this taxon, even if gut microbes may provide important nutritional services. For example, Ravenscraft et al. (2019) sampled adults of almost 300 tropical butterfly species, and found that communities of gut bacteria and fungi were a subset of those found in the food. This suggests that the adult gut acquires microbes from the plant but selects those that may aid in digestion. This speculation was indirectly supported by means of culture-based assays that revealed that the microbiomes of frugivores and nectarivores differed in their catabolic abilities. Microbial symbionts may also detoxify plant secondary metabolites in larvae as found, for example, for the degradation of phenolic glycosides in the gypsy moth *Lymantria dispar* (Mason et al., 2014), proteinase inhibitors in the velvet bean caterpillar *Anticarsia gemmatalis* (Pilon et al., 2013; Visôtto et al., 2009), and potentially in cycad feeding specialists as discussed in the Coleoptera section (Salzman et al., 2018).

Relative to caterpillars, adult butterflies were also shown to have richer bacterial microbiomes (Hammer et al., 2019a; Ravenscraft et al., 2019). A large screening of microbiome from wild-caught butterfly species across the Heliconiini revealed that closely related butterfly lineages harboured similar microbiomes (Hammer et al., 2019a). A correlative analysis between microbial community similarities and host phylogenetic distances showed moderate congruent topologies of the butterfly phylogeny with the microbiome dendrogram. For instance, pollen-feeding *Heliconius* had distinct, albeit low diversity bacterial communities relative to non-feeding butterflies. Although host-microbe co-diversification could be a plausible explanation for these observations, Hammer et al. (2019a) proposed that these differences were due to insect guts filtering out bacterial lineages depending on the diet of the host. Similar results were shown by Ravenscraft et al. (2019) in a study comprising over 50 different neotropical butterfly species. The role of microbes in adult butterflies thus remains little understood, but it can range from preventing pathogen colonization to direct antiparasite interactions. These studies highlight that in Lepidoptera, microbiomes shift in their composition and diversity across the different life stages and emphasizes the need to separately look at the community composition in relation to diet and development.

If Lepidopteran microbiomes are assembled from microbes in the local environment, from which microhabitats do these microbes arise? Soil microbes are among the most abundant and diverse group of organisms on Earth (Delgado-Baquerizo et al., 2018), and Hannula et al. (2019) showed that the entire microbiome of caterpillars of the cabbage moth, *Mamestra brassicae* (Fig. 2P), was affected by bacteria and fungi largely present in the soil and not in the host plant they were feeding on. These findings support prior discoveries that soil legacy effects have impacts on plant growth and plant-insect interactions (Pineda et al., 2017). Yet, Hannula et al. (2019) conducted their experiments under artificial conditions in a greenhouse with a moth strain reared for several years under laboratory conditions. Further studies need to address whether soil microbes affect aboveground insect microbiomes under more natural conditions, and how widespread such soil legacy effects are. This may be potentially more common in polyphagous caterpillars like *M. brassicae* that are often in direct contact with the soil because they often move up and down the plant to switch host plants until they pupate. On the contrary, specialist butterfly species like *Pieris brassicae* (Fig. 2Q) usually stay on the same plant throughout most of larval development eventually moving up to feed upon flowers (Lucas-Barbosa et al., 2013). It would be interesting to understand whether such butterfly species acquire microbes from the soil as well but maybe indirectly via the plant as found in the honeybee example discussed above (Kim et al., 2019).

3.3.1 Symbionts modulate induced plant defences in Lepidoptera

Probably one of the first examples of plant physiology manipulation by insect symbionts was found in the Gracillariidae leaf miner moth *Phyllonorycter blancardella*. This moth carries the bacterium *Wolbachia*, which aids its host by manipulating plant hormones called Cytokinins (Giron et al., 2013). In autumn, such phytohormone manipulation keeps the leaf surrounding the insect photosynthetically active and green, in otherwise decaying leaves (i.e. the 'green island effect'). A more recent study revealed that acquisition of *Wolbachia* in two lepidopteran lineages (including 60 species from the Gracillariidae family) was associated with the green island phenotype (Gutzwiller et al., 2015). This study is one of the few exploring the role of symbionts in manipulating plant physiology through use of a phylogenetic framework.

Similar to the whitefly *B. tabaci* and some beetle species, oral secretions of the fall armyworm, *Spodoptera frugiperla*, and corn earworm, *Helicoverpa zea* (both Noctuidae), have also been found to modulate plant defences. While for the Colorado potato beetle the effect found was clearly beneficial, for these moths the benefit is less clear. Acevedo et al. (2017) identified a whole suite of bacteria from oral secretions of *S. frugiperla*, and using culture-dependent methods tested their effect on plant defences. Results did not point to a clear benefit for the insect because different bacterial species had varying effects on different markers of defence-related genes. A similar result was found in a later study exploring the presence of phytohormones in the saliva of this same moth (Acevedo et al., 2019). Studies with *H. zea*, however, clearly showed that gut-associated bacteria often betray their host as they triggered insect-specific plant defences (i.e. enzyme polyphenol oxidase and genes regulated by jasmonic acid) (Wang et al., 2017). Bacteria that reside in the gut can also become the target of plant defences. When feeding on highly resistant maize plants with increased concentrations of chitinases and large trichomes (a type of physical plant defence), *S. frugiperda* usually shows reduced fitness. A study by Mason et al. (2019b) revealed that these effects may be partially explained by plant defences weakening protective layers of the insect gut thus allowing resident bacteria to colonize the hemolymph and become pathogenic.

Defences of plants can be primed by a preceding environmental stimulus, such as herbivore egg deposition, reliably indicating upcoming herbivorous feeding damage (Hilker et al., 2015). Paniagua-Voirol et al. (2020) investigated whether bacteria present on eggs of cabbage white butterflies, *Pieris brassicae*, (Fig. 2Q) are involved in eliciting an egg-mediated enhancement of plant defences against caterpillars. When treating butterflies with antibiotics, the egg-mediated plant response diminished: both caterpillar performances and transcriptional responses of the plant were similar to control plants that did not receive egg deposition. However, culture-independent quantification of bacteria revealed very low and inconsistent bacterial abundances on the eggs. Despite these results, bacteria were four times more abundant in female butterflies than in eggs, thus confirming that adult butterflies have very diverse microbiomes (Hammer et al., 2019a; Ravenscraft et al., 2019). The present study was done with butterflies cultured for numerous generations under laboratory and therefore more sterile environmental conditions. Previous studies on different moth species comparing field and laboratory populations have shown that laboratory reared insects are depauperate and largely dominated by a few bacterial strains (Belda et al., 2011; Staudacher et al., 2016; Xiang et al., 2006). More studies with Lepidoptera directly obtained from the field are thus likely to provide novel and exciting discoveries in this group.

3.4 Diptera

One of the best examples of co-speciation between dipterans and symbionts is that of ambrosia gall midges (Cecidomyiidae) and their fungal associate *Botryosphaeria dothidea*. In this group, fungal spores are deposited into plant tissues along with insect eggs, and hyphae line the inner walls of the gall providing food for the larvae and protection against natural enemies (e.g. Rohfritsch, 2008). Joy (2013) performed a large phylogenetic analysis of midge-plant associations comprising a total of 351 genera of gall-inducing midges and found that an association with the fungus accelerated diversification in this group and broadened the number of host plants exploited. In particular, symbiotic lineages were found to be more than 17 times more diverse than non-symbiotic ones, and had undergone a sevenfold increase in the number of host plant species used.

The number of species of herbivorous dipterans that make galls, however, is a minority, as most species feed on decaying plant material. Symbiosis with bacteria and yeasts in this guild has been studied in pest species in the families Drosophilidae and Tephritidae (Fig. 2R and S), and particularly in the model species *Drosophila melanogaster*. In laboratory-reared *D. melanogaster*, even if some microbes are resident (Ma and Leulier, 2018), the composition of microbial communities (both bacteria and yeasts) is usually similar between the food and the insect (reviewed in Broderick and Lemaitre, 2012). Bacterial symbionts are likely to be acquired by the larvae from the environment, and the microbes introduced to larval food sources by adult flies where they metabolize or detoxify products that are then fed upon by developing larvae. In wild *Drosophila*, however, gut microbes are usually different from that of the food source (e.g. Wong et al., 2015), which questions whether some resident microbes exist in this group. *Drosophila* is a diverse genus of flies, and whether symbionts have played a role in their diversification is a question that has been recently debated. Using six species obtained from laboratory cultures Brooks et al. (2016) pinpointed microbes as the driving force of *Drosophila* evolution because the authors found a strong congruence between the fly phylogeny and the dissimilarity matrices of bacterial communities. In a more recent study with four mushroom-feeding species of wild *Drosophila*, Martinson et al. (2017b) challenged this view. In this later report, the authors found that the microbiota of the different species were very similar, probably reflecting an effect of the exploited ecological niche and not the phylogeny of the host. This same result was found in a group of cactus-feeding *Drosophila* species that are endemic in the Sonoran desert (Martinson et al., 2017a; Starmer and Fogleman, 1986).

Tephritids are a group of dipterans comprising important pests like the oriental fruit fly (*Bactrocera dorsalis*) and the Mediterranean fruit fly (*Ceratitis capitata*), the latter species being the model species for the study of symbiosis in the Tephritidae family. As with *Drosophila*, tephritids carry yeasts and bacteria in their guts that are transferred to the fruit by females during oviposition where they proliferate eventually causing decay (reviewed in Lauzon, 2003). The bacteria found in *C. capitata* mostly comprise *Enterobacter*, *Klebsiella* and *Pectobacterium*, some of them with the ability to fix atmospheric nitrogen and to degrade pectin, thereby playing an important role in fly nutrition. The tight interaction between the fly and some of these bacteria is revealed by the presence of an oesophageal bulb in the fly capable of hosting the microbes (Sacchetti et al., 2008). Some Tephritidae pests are currently controlled using the sterile insect technique and mass releases of parasitoids. These techniques require rearing large numbers of flies and research on fly symbionts aims at improving this method (Ras et al., 2017).

Many fly species are pests that cause sour rot in fruits. An important question in these species is whether microbes that are carried by adults and deposited in fruits during oviposition are the main players inducing fruit decay. In an experiment inoculating grape fruits with different microbial symbionts of *Drosophila* sp. it was found that sour rot requires both the insect and its associated consortium of microbes, particularly yeasts and acetic acid bacteria (Barata et al., 2012). Another important question in this guild of flies is how rotting microbes penetrate the fruit. Some species can only colonize and spread rotting in already damaged fruits, while others are capable of attacking healthy fruits. This is the case of the spotted wing drosophila, *D. suzukii*, an emerging global pest. Although this fly can also cause sour rot in fruits (Ioriatti et al., 2018), whether vertically-transmitted symbionts play a role in allowing it to attack healthy fruits is not yet known. A recent study, however, demonstrated that even if *D. suzukii* is not capable of producing extensive fruit rot in vineyards, the wounds it produces to healthy fruits opens a window of susceptibility to more damaging drosophilids like *D. melanogaster* (Rombaut et al., 2017). This study highlights the importance of the community context to understand the role of fly symbionts in the biology of key pests.

4. The impact of insect symbionts in a changing world: Outlook and perspective

4.1 Insect symbionts in a warmer world

There is now unequivocal evidence that the climate is changing. One of the clearest trends is that mean global temperatures increased on average 0.6 °C during the last century, and particularly during the second half (IPCC, 2019). Recent reviews suggest that symbionts will critically modulate insect responses to global warming by either exacerbating extinctions or by buffering them (Corbin et al., 2017; Renoz et al., 2019; Thierry et al., 2019). Global warming may disrupt the symbiosis between insect hosts and their symbionts (symbiosis meltdown) or symbionts may buffer any negative effects of warming on the host (adaptation).

The phenomenon of symbiosis meltdown has been well described in some taxa like corals (Toby Kiers et al., 2010). In insect herbivores, there are few good examples in which increased temperatures lead to the death of the insect via negative effects on obligatory bacterial symbionts. These examples include pea aphids, the stink bug *Nezara*, the whitefly *Bemisia tabaci*, mealybugs and weevils (recently reviewed in Corbin et al., 2017; Renoz et al., 2019; Thierry et al., 2019; Wernegreen, 2012). In all these examples, experiments have shown that experimentally increasing temperatures led to symbiont mortality and concomitantly to the death of the insect host (i.e. the symbiont is the thermal 'weak link', Corbin et al., 2017).

A symbiosis meltdown does not necessarily result in extinction of both species. Renoz et al. (2019) provide several examples in which insects have lost a weak symbiotic partner by incorporating the microbial function into the host's genetic repertoire via horizontal gene transfer. Moreover, if the symbiont is facultative, the symbiont partner could be temporarily lost and subsequently re-acquired from the environment. This may be possible in groups like stink bugs that commonly acquire mutualistic bacterial symbionts from the soil each generation (e.g. Kikuchi et al., 2012). The plant host can also influence these interactions, for example by protecting insect symbionts from heat stress on their surfaces or even inside their tissues. The colonization of plant tissues by insect symbionts has been recently demonstrated in phloem-feeding insects as a means for horizontal transmission (reviewed in Chrostek et al., 2017) and can have additional benefits with increasing temperatures. A recent study showed that an important part of the microbiota of the moth *M. brassicae* is acquired from the soil (Hannula et al., 2019). Since soil may buffer temperature variations, plant roots could be thus an important reservoir of insect symbionts during heat waves. A few facultative endosymbionts have been found to protect their hosts against heat shocks (e.g. above 35 °C), and it is likely that associations with these bacteria will be favoured on a warmer planet. The aphid symbiont *Serratia symbiotica* was the first found to confer heat resistance, but similar roles have been reported for *H. defensa* in pea aphids and the whitefly *B. tabaci*, and also for *Rickettsia* and *Fukatsuia* in pea aphids (Oliver et al., 2010; Thierry et al., 2019). As far as we are aware, gut symbionts with the ability to protect insects against heat shock have not been identified.

Global warming may also make insects less protected by symbionts because at increased temperatures defensive symbionts may be lost, or become more costly. For example, the symbiont *H. defensa* protects its aphid hosts from parasitoids under normal temperatures, but fails at doing so when the host is exposed to heat shock (Bensadia et al., 2006; Doremus et al., 2018). The host plant can again alter these interactions. A recent study by Sochard et al. (2019) showed that the defensive symbiont *H. defensa* defended aphids against a parasitic wasp when these aphids were originally collected from *Medicago sativa* plants, but not from *Ononis spinosa* host plants. It is thus feasible that global warming alters not only aphid persistence on particular host plants, via effects on the symbionts, but also the associated community of natural enemies. Some natural enemies like parasitic wasps also carry symbionts that are needed for successful parasitism (Dicke et al., 2020). Future studies are therefore needed to explore this question, as symbiosis meltdown in natural enemies may release herbivore populations from top-down control and trigger insect outbreaks with cascading effects on plant communities.

4.2 Insect symbionts mediate the transmission of plant viruses

Many insects are important vectors of plant viruses and the first study to associate insect symbionts with virus transmission was in the early 1990s by Van den Heuvel et al. (1994). They were studying the transmission of potato leafroll virus by the aphid *Myzus persicae*, and found the virus had a high affinity for the protein symbionin (now named GroEL) that is produced by the aphid primary symbiont (*Buchnera aphidicola*). Subsequent research has questioned its role for circulating viruses, as *Buchnera*-GroEL was localized in the bacteriocytes rather than the haemolymph, gut or fat body, which would be needed for circulative virus transmission (reviewed by Pinheiro et al., 2015). Yet, the observation of a similar effect from a GroEL protein of the secondary symbiont *H. defensa* in whiteflies, and compelling experimental data that *H. defensa* enhances viral transmission (Bello et al., 2019; Guo et al., 2014; Hong et al., 2017; Su et al., 2013a), still shows we do not yet have a full understanding of this.

Direct interactions between insect symbionts and viruses are, in no doubt, important for circulative and persistent viruses that stay with the insect its whole life. However, all viruses (non-persistent, semi-persistent, or persistent) can also manipulate the insect to increase the chance of transmission. Such indirect interactions can occur through influencing host-plant selection (Ingwell et al., 2012), the production of winged individuals for dispersal (Hodge and Powell, 2010), and increased feeding on virus-infected plants (Angelella et al., 2018). All of these viral effects can also be influenced by insect symbionts (Frago et al., 2012). For example, aphids probe plants as a ‘taste test’ when finding new suitable host plants, and this probing activity can be increased both by *H. defensa* symbiont infection and by the *watermelon mosaic virus* infecting pumpkin plants and cowpea aphids (*Aphis craccivora*) feeding on it (Angelella et al., 2018). For the symbiont effect, an increased amount of probing when infected by *H. defensa* could act to further reduce plant defences which can also benefit successful virus transmission. A plant’s induced defence response to virus infection can act to reduce the fitness of both virus and insect, but it is in the virus’ best interests to maximize the fitness of the insect host. In this way persistent viruses and insect symbionts could be both considered as mutualists to the insect because they fight the plants immune system, minimize pathogenic effects in the vector and ultimately increase insect fitness (Pinheiro et al., 2015). Another challenge where both virus and bacterial symbionts need to benefit the host is in response to abiotic stress. Hong et al. (2017) showed that increasing ozone concentrations further enhanced *tomato yellow leaf curl virus* transmission among plants by whiteflies (beyond any symbiont effect). They concluded that the effect occurred through ozone-stress acting to decrease plant resistance and immune responses to the virus, and hypothesized that environmental stress, mediated through changes in the insect protective microbiome, resulted in indirect effects on insect 'endurance'.

Transmission of viruses among insect hosts does not just occur as a vector moves from plant to plant (horizontal transmission), but also from parent to offspring through vertical transmission (Jia et al., 2018). This is also the main way in which bacterial symbionts are transmitted across insect hosts, and therefore this route is important when considering the interactions between viruses and insect symbionts. Green leafhoppers (*Nephotettix cincticeps*) host two obligate symbionts (*Sulcia* and *Nasuia*) and are common pests of rice fields, transmitting various plant viruses. Two recent studies have investigated the role of these symbionts on the vertical transmission of *rice dwarf virus* (Jia et al., 2017; Wu et al., 2019). Both studies show that the virus uses the symbionts as a vehicle to transfer to the next generation, by binding to the outer envelope of *Sulcia* and moving through the envelope to enter the periplasmic space of *Nasuia*. For both, as the symbiont is transmitted to the offspring, so is the virus. It is unknown if similar mechanisms may occur in other commonly studied symbiont systems like whiteflies and aphids.

4.3 Insect symbionts in agriculture, beyond effects on natural enemies

Future outlooks for managed systems under climate change predict increased impacts of pests and disease (IPCC, 2019). Many insect symbionts act to benefit the survival (nutrition, resistance to biotic and abiotic stress) and dispersal (host-plant use or production of dispersal morphs) of their insect hosts. The impact of insect-symbiont interactions on pest biocontrol via natural enemies has been recently explored in the realm of food web interactions (McLean et al., 2016), the spread of symbiont-mediated natural enemy resistance in closed greenhouse systems (Vorburger, 2018) and how plant biodiversity can mitigate symbiont-mediated resistance in open crop fields (Zytynska and Meyer, 2019).

Beyond nutritional and protective services, insect symbionts in agriculture can also benefit their host by detoxifying and degrading chemical pesticides. In natural systems, a host's bacterial community can help them feed on highly-defended plant hosts by transforming secondary plant metabolites (Dillon and Charnley, 2002; Howe et al., 2018). Adaptation of this to combat the effects of sprayed chemical pesticides leads to symbiont-mediated pesticide resistance (van den Bosch and Welte, 2017) and has been demonstrated in a number of pest species. For example, a *Burkholderia* symbiont of the bean bug *Riptortus pedestris* (Fig. 2G), a major pest of soybean in Japan, can degrade the organophosphate fenitrothion (Kikuchi et al., 2012), *Bacillus cereus* in the guts of diamondback moths, *Plutella xylostella*, can breakdown indoxacarb and acephate (Ramya et al., 2016; van den Bosch and Welte, 2017), and the gut symbiont *Citrobacter* of tephritid fruit fly pests (Fig. 2S) can degrade trichlorphon (Cheng et al., 2017). The increasing focus on biopesticides should therefore take into consideration the adaptive potential not only of the host insect being targeted but also of the symbiotic bacteria that are hosted by the insect. Moreover, horizontal gene transfer can also transfer resistance traits from bacteria to the host (Husnik and McCutcheon, 2018), which means that even if the symbiotic relationship breaks down the insect will still maintain the resistance trait.

Targeting insect symbionts for pest biocontrol has strong potential and would be particularly valuable against symbionts synthesizing essential nutrients for their host, e.g. obligate symbionts of insects feeding on vertebrate blood, plant sap, and deadwood (Arora and Douglas, 2017; Popa et al., 2012). If an obligate symbiont can be eliminated from the insect host, the insect will suffer from reduced nutrition leading to reduced reproduction, population growth and survival. Research to identify appropriate genes that can be targeted for deletion by RNA interference (RNAi) to reduce pest populations is ongoing (Niu et al., 2018). For example, the deletion of *amiD* and *ldcA1* genes caused the symbionts to be attacked as another pathogen, hindering symbiont acceptance by the insect host with knock-on effects for host fitness (Chung et al., 2018). However, there will be a selective pressure for insect host to evolve to regain this symbiosis or to evolve a different strategy to compensate for this loss (e.g. with other bacterial symbionts) and over time we might expect such targets to reduce in effectiveness. Despite these issues, in the short-term and with sufficient management control for the use of such methods this is a promising area for pest control. In the United-States, four transgenic insecticidal proteins are registered for corn rootworm management and the latest involves use of

RNAi to silence important genes influencing cornworm development (reviewed by Fishilevich et al., 2016). The use of RNAi allows a large number of different genes to be targeted, and as we learn more about the molecular basis of ecological interactions more targets will be uncovered. Targeting a larger number of genes with RNAi lowers the probabilities that insects adapt to and overcome such transcriptional inhibitions. In mosquitoes, the deletion of an outer-membrane gene (ompA) of a dominant gut bacterial symbiont using CRISPR/Cas9 impaired biofilm formation and reduced gut colonization (Hegde et al., 2019). While this mutation did not influence host fitness, it is thought to have implications for vector competency where this method of gene editing can be used for site-specific integration of genes into the symbiont that reduce the spread of viral diseases. Targeting insect symbionts that are also involved in virus transmission could benefit plant production through simultaneous reduction of pests, vectors and disease (Chuche et al., 2017). All these methods would need to be implemented as part of an integrated pest management plan that involves a multifaceted approach to pest control using a combination of pest abundance surveys, increased recruitment of natural enemies, alongside use of symbiont- or insect-targeted biopesticides.

5. Conclusion

Our systematic review of microbial symbiosis in herbivorous insects shows that not all insect orders rely equally on microbial symbionts to thrive on plants, and that there is much variation with respect to symbiont localization in hosts (e.g. guts versus hemocoel). Symbiont-dependent insect species are particularly present in Hemiptera, which evolved to feed on low-nutrient plant sap with the help of obligate endosymbionts that have been vertically transmitted for millions of years. Other orders seem to rely on genes acquired from microbes via ancient lateral gene transfers, and have developed distinct feeding niches without depending on costly symbioses. When present in these lineages, gut microbes were often shown to be transient and taken up from the environment, such as the host plant or the soil. In accordance with Hammer et al. (2019b) who states that animals vary greatly in their degree of reliance on microbial symbiosis, we see this diversity of interactions, particularly in some diverse orders like Lepidoptera or Coleoptera. In these orders, some species use the highly diverse microbiomes of their food plants to filter them into their guts and to harbour them for a certain period of time rather than let them become resident.

Further large-scale screenings of herbivorous species and their food plants should shed light on how common this rather loose type of association between microbes and their insect hosts is. Although the hemimetabolous Hemiptera order is largely relying on obligatory microbial symbiosis, we did not find such a close relationship between symbionts and hosts in the other three orders Orthoptera, Phasmatodea and Thysanoptera. Instead, these harbour transient gut symbionts and/or rely on laterally transferred genes from microbes. Despite the radical changes and harsh conditions during metamorphosis numerous holometabolous species vertically transmit their symbionts and show close associations with both intra- and extracellular symbionts. Some species maintain the same symbiont in larvae and adults but harbour them in different tissues, while in other species, communities differ between larvae and adults, largely because of variations in habitat use or diet.

The role that symbionts can play in future scenarios of global warming has a strong potential to change insect population dynamics and ultimately the persistence of insect populations, the structure of their communities, and the services they provide. Global warming and habitat loss are triggering massive species extinctions, and these extinctions maybe either exacerbated or buffered by symbionts as they can be the weaker link in insects, or protect against environmental stress. Symbiont effects can change the pest status of some insects, triggering outbreaks in some species, but also reducing the densities of others. However, this also gives rise to the potential to better control these pest populations by manipulating the symbiont relationship with their host. The last few years have revealed an unprecedented decline in insect abundance in many natural ecosystems (Hallmann et al., 2017). It is likely that a subset of this loss has been mediated by impacts on insect symbionts. Populations of rare or endangered species, for example, can be reduced if these species carry symbionts that are susceptible to increased warming. Extinctions can also be triggered if communities become dominated by few species that carry symbionts that allow them to better sustain increased temperatures. Habitat loss is an important element challenging insect populations. When the amount of habitat is reduced, species persistence is jeopardized because resources are of lower quality and critical resources can be missing (Samways et al., 2010). These critical resources can be symbionts, an effect that can be particularly dramatic for species that rely upon environmental symbionts, acquired anew at every generation. In the last 20 years, the steadily growing literature investigating the ecology and evolutionary potential of insect symbionts has enabled the development of strategies to transfer basic science to commercialisation. We are now at an age where we can bring together ecology, evolution and biotechnology to benefit the management of cropping systems, and to better understand the role of microbes in natural insect populations to protect endangered or beneficial species.

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ARTICLE

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Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles

Enric Frago[1,2], Mukta Mala[1], Berhane T. Weldegergis[1], Chenjiao Yang[1], Ailsa McLean[3], H.Charles J. Godfray[3], Rieta Gols[1] & Marcel Dicke[1]

Plants respond to insect attack by releasing blends of volatile chemicals that attract their herbivores' specific natural enemies, while insect herbivores may carry endosymbiotic microorganisms that directly improve herbivore survival after natural enemy attack. Here we demonstrate that the two phenomena can be linked. Plants fed upon by pea aphids release volatiles that attract parasitic wasps, and the pea aphid can carry facultative endosymbiotic bacteria that prevent the development of the parasitic wasp larva and thus markedly improve aphid survival after wasp attack. We show that these endosymbionts also attenuate the systemic release of volatiles by plants after aphid attack, reducing parasitic wasp recruitment and increasing aphid fitness. Our results reveal a novel mechanism through which symbionts can benefit their hosts and emphasise the importance of considering the microbiome in understanding insect ecological interactions.

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[1] Laboratory of Entomology, Wageningen University, P.O. Box 16,6700AA Wageningen, The Netherlands. [2] CIRAD, UMR PVBMT, Saint-Pierre, La Réunion F-97410, France. [3] Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK. Correspondence and requests for materials should be addressed to E.F. (email: enric.frago@cirad.fr)

Associations between microbial symbionts and multicellular eukaryotes are widespread in nature and many organisms rely on symbionts for a variety of functions important for their survival and reproduction[1]. Acquisition of symbionts can be key innovations that allow diversification into unexploited adaptive zones. Many insects depend on bacterial symbionts to provide essential nutrients that are otherwise missing from their diets[2]. These types of symbionts are termed obligate as they are essential for survival. There is increasing interest in the role played by facultative symbionts that, while not essential for survival or reproduction, provide important services for their hosts. In the last 20 years, the discovery that many facultative symbionts help protect their hosts from natural enemies has transformed our understanding of how insect symbionts affect the interactions between their hosts and higher trophic levels[3]. Symbiont-conferred protection against pathogens, parasitic wasps and predators has been demonstrated in a variety of different species, and recent evidence suggests that they can also help herbivores overcome specific induced defences mounted by plants in response to insect attack[4]. For example, the whitefly *Bemisia tabaci* and the Colorado potato beetle *Leptinotarsa decemlineata* carry facultative symbionts that manipulate host plant physiology through salivary effectors that attenuate induced defences to the benefit of their hosts[5, 6].

Natural enemies of herbivorous insects commonly use volatile chemical cues to locate their often concealed hosts or prey in the structurally complex environment which they inhabit[7]. Mutualistic symbionts could affect the likelihood of their hosts' discovery in two ways. First, they might produce "infochemicals" that attract natural enemies. For example, bark beetles carry symbiotic fungi which they use to digest wood, but the symbiont also releases volatiles that attract parasitic wasps[8, 9]. Here the nutritional benefit provided by the symbiont may be counteracted by increased attraction to natural enemies, something that may lead to the loss of the microbial partner in the host population when natural enemy pressure is high. Second, symbionts may interfere with the plant's ability to attract its herbivore's natural enemies so benefiting the host. Plants often respond to herbivore attack by releasing a specific blend of volatiles that attract the insect's natural enemies (so-called 'bodyguard recruitment')[10]. We do not know whether the presence of facultative symbionts interferes with the induction of this indirect herbivore-defence mechanism.

Here we studied symbiont-herbivore-natural enemy interactions on the pea aphid (*Acyrthosiphon pisum*) feeding on the broad bean *Vicia faba*. This plant is known to respond to pea aphid attack by releasing volatiles that attract the parasitic wasp *Aphidius ervi*, and olfactometer and biochemical studies have identified the specific volatiles that are involved in wasp attraction[11–14]. These studies have also shown that parasitic wasps are attracted to blends of volatiles rather than to individual compounds[15], yet the effective blend has not yet been elucidated for any parasitoid species. Pea aphids are associated with at least eight different facultative symbionts[16–18] including *Hamiltonella defensa*, many strains of which increase survival after parasitic wasp attack[19, 20]. Using olfactometer choice experiments, we investigated whether pea aphids benefit from carrying the symbiont *H. defensa* by influencing the release of volatiles and reducing parasitic wasp recruitment, and whether any effect on volatile release was localised to the site of aphid attack or systemic. There are costs to carrying the symbiont[21–23] and in further experiments we assessed for any effect of aphid vigour on the plant's response. To do this, we measured aphid offspring (a good measure of vigour), and performed experiments infesting bean plants with a varying number of aphids. Different populations (or biotypes) of pea aphid are adapted to different host plants, though all feed on *V. faba*, which is considered a "universal" host[24]. Most of our experiments were performed on *V. faba* and we asked whether the natural host plants (*Ononis spinosa* and *Lotus pedunculatus*) of two different biotypes originally collected on these plants showed the same response. We carried out experiments in microcosm cages to test whether differential recruitment of parasitic wasps translated into reduced parasitism. The blend of volatiles present in the 'headspace' around plants fed upon by symbiont-carrying and uninfected aphids was characterised to identify the mechanistic basis of the effect we observed.

Finally, we carried out a more limited set of olfactometer experiments to test whether carriage of four other endosymbionts affected recruitment of the parasitic wasp *A. ervi*. The species we studied were (i) *Regiella insecticola*, which typically protects *A. pisum* from specialist pathogenic fungi but not parasitic wasps[25]; (ii) *Spiroplasma* sp. which, depending on the particular isolate, may or may not confer protection against wasps[26, 27]; (iii) *Serratia symbiotica*, which also shows some strain-specific parasitic wasp protection[20] and protects aphids from heat shocks[28]; and (iv) *Rickettsiella* sp., which is less well characterised but is known to influence aphid body colour and hence possibly attraction to natural enemies[29].

Our study shows that plants infested with aphids carrying different symbiont species and strains are less attractive to the parasitic wasp *A. ervi* through systemic changes in herbivore-induced plant volatiles, ultimately reducing parasitic wasp recruitment and increasing aphid fitness. We demonstrate this with behavioural experiments, but also analysing volatile blends. Relative to plants fed upon by symbiont-free aphids, blends of plants fed upon by aphids carrying the symbiont *H. defensa* are different and total emissions are lower. Our results reveal a novel mechanism by which insect symbionts protect their hosts through manipulation of induced defences mounted by plants in response to insect attack.

Results

Effect of symbiont *H. defensa* on parasitic wasp attraction. Across a large set of experiments with different symbiont strains and plant species, and using choice experiments, parasitic wasps were significantly less likely to be attracted to plants that had previously been fed on by aphids carrying *H. defensa* compared to plants fed on by aphids that carried no secondary symbionts (Fig. 1, with statistical results in the legend). This was true in experiments where the comparison was between natural aphid-symbiont associations and the same aphid clone from which the symbiont had been removed using antibiotics (Fig. 1a, columns i–iii), and in experiments where naturally secondary symbiont-free aphids were compared to aphids which had received the symbiont through microinjection (Fig. 1a, columns iv–v). The preference was not affected by wrapping the leaf on which the aphids had fed in aluminium foil (Fig. 1a, column vi), which excludes the possibility that parasitoids are attracted to any chemical deposited by the aphid and shows that the attractant volatiles are produced systemically by the plant. The parasitoids still showed a preference for plants that had been fed on by aphids not carrying *H. defensa* when the densities of aphids with the symbiont were doubled (Fig. 1b). This means that the reduced attraction is not due to diminished damage caused by aphids carrying a potentially costly symbiont, a conclusion confirmed by the lack of a correlation between wasp preference and aphid vigour as estimated by relative progeny production (Fig. 2a, Supplementary Table 1). Two of the natural symbiont-aphid associations involved aphids belonging to biotypes associated with *Ononis spinosa* and *Lotus pedunculatus* and which had been collected on these host plants. We found the same wasp

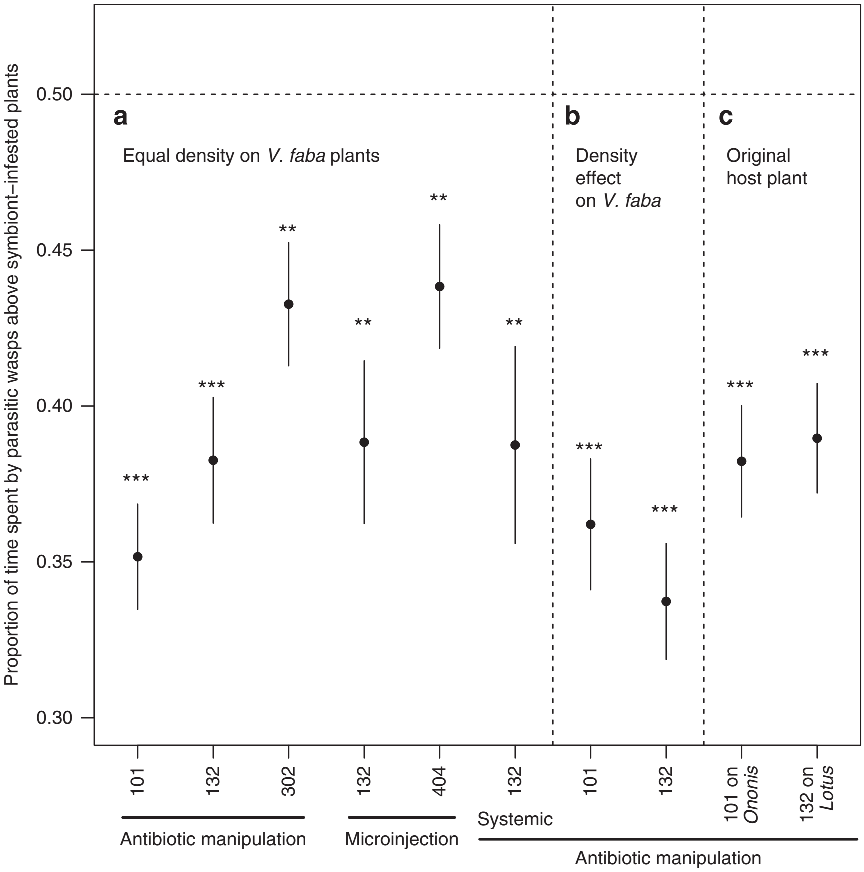

Fig. 1 Parasitic wasp preference for volatiles from Fabaceae plants infested with aphids with or without the endosymbiont. Parasitic wasp (*Aphidius ervi*) response to volatiles emitted by plants infested with aphids (*Acyrthosiphon pisum*) with or without the endosymbiont *Hamiltonella defensa*. For each test, the bars show standard errors and the asterisks the significance of the deviation from no choice (*t*-test: $^{**}p < 0.01$; $^{***}p < 0.001$). **a** Tests where *Vicia faba* plants had been induced by 20 wingless aphid females each ($n = 10$; column i—cured 101: $t_9 = -8.78$, $p < 0.0001$; ii—cured 132: $t_9 = -5.82$, $p = 0.0001$; iii—cured 302: $t_9 = -3.41$, $p = 0.0039$, iv—microinjected 132: $t_9 = -4.27$, $p = 0.0010$; v—microinjected 404: $t_9 = -3.11$, $p = 0.0062$, vi—cured 132: $t_9 = -3.56$, $p = 0.0031$). **b** Tests with *V. faba* plants induced with double the number of symbiont-bearing compared to symbiont-free aphids ($n = 10$; column vii—cured 101: $t_9 = -6.57$, $p < 0.0001$; viii—cured 132: $t_9 = -8.73$, $p < 0.0001$). **c** Tests with the original host plants *Ononis spinosa* ($n = 10$; column ix—cured 101, $t_9 = -6.58$, $p < 0.0001$) and *Lotus pedunculatus* (column x—cured 132, $t_9 = -6.28$, $p < 0.0001$). Microinjections were performed into an aphid clone naturally lacking any secondary symbiont, and which was collected on *Lathyrus pratensis*. Note that the same symbiont strain and aphid clone may have been used in different tests

preferences when the experiments with these clones were repeated on the natural host plant as we had when working with *Vicia faba* (Fig. 1c).

Effect of symbiont *H. defensa* on parasitic wasp attack. We used population cage experiments to explore whether the reduced attractiveness to parasitic wasps of plants fed upon by aphids carrying *H. defensa* translates into lower rates of parasitism. Parasitism was assessed by placing 'sentinel aphids' belonging to two secondary symbiont free clones that can be recognised by a colour polymorphism on the plants immediately before the introduction of parasitoids. This polymorphism had no effect on wasp parasitism (0.80 ± 0.05 [SE] vs. 0.75 ± 0.06; paired *t*-test: $t_{14} = 1.26$, $n = 15$, $p = 0.2298$). Aphids on plants previously infested by symbiont-carrying aphids were parasitized significantly less often than the alternative (0.62 ± 0.04 [SE] vs. 0.94 ± 0.01; paired *t*-test: $t_{14} = 7.73$, $n = 15$, $p < 0.0001$).

Effect of symbiont *H. defensa* on plant volatile emission. Volatile compounds were collected from plants previously attacked by aphids carrying or not carrying *H. defensa* and analysed using gas chromatography mass spectrometry. Overall we found 66 volatile compounds (Supplementary Table 2) and a Principal Least Squares Discriminant Analysis (PLS-DA) showed a significant difference in the volatile composition of the headspace of the two types of plant (Fig. 3a; NMC = 0.1778, $n = 9$, $p = 0.0151$). As revealed by VIP scores (variable importance in projection), treatment separation was chiefly due to 24 compounds. Among these compounds, nine were significantly more abundant in the treatment with no *H. defensa*, while we did not find any compound to be significantly more abundant in the symbiont treatment (Figs. 3b, 4, Supplementary Table 2). Of the 66 volatile compounds, 55 had mean concentrations that were greater in plants with symbiont-free aphids than in those where the symbiont was present. Overall, total emissions were significantly lower in plants fed upon by aphids carrying the symbiont compared with symbiont-free insects (sign test $S = 55$, $n = 66$, $p < 0.0001$).

The effect of other symbionts on parasitic wasp recruitment. We explored the effect of feeding by aphids carrying other facultative symbionts on wasp preferences in choice experiments. Four experiments were carried out with *Regiella insecticola*, two using natural associations and two in which different symbiont strains were introduced into an aphid clone that carried no

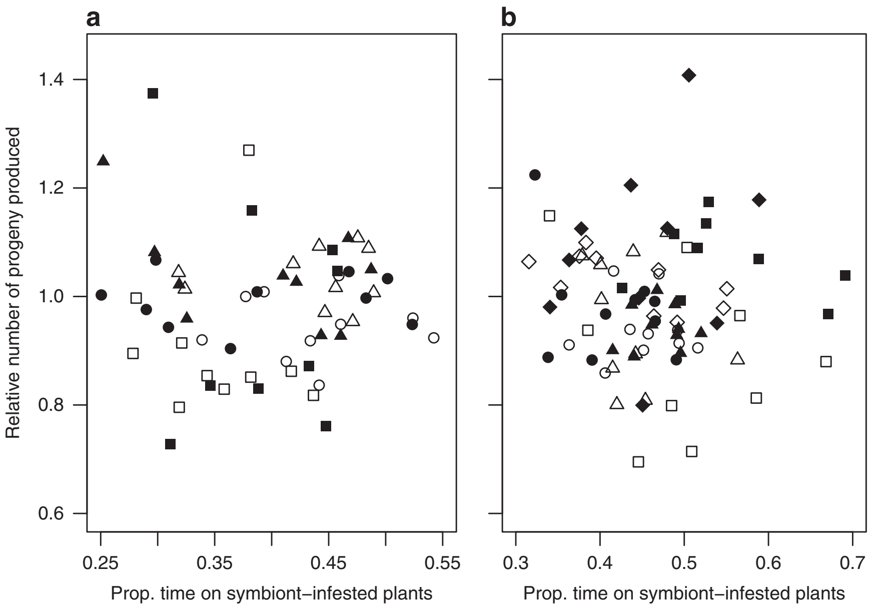

Fig. 2 Relationship between aphid vigour and parasitic wasp response. Relationship between the relative number of progeny produced by symbiont-carrying and symbiont-free *Acyrthosiphon pisum* aphids on *Vicia faba* plants, and the mean proportion of time spent by parasitic wasps *Aphidius ervi* above symbiont-infested plants. **a** Tests with the symbiont *H. defensa*. These test include natural symbiont infections where the symbiont was removed with antibiotics in strains 101 (empty squares), 132 (solid squares) and 302 (empty triangles); artificial injections of strains 132 (solid triangles) and 404 (empty circles); test for systemic release of volatiles with an artificial injection of strain 132 (solid dots). **b** Tests with the other symbiont species. These include natural *Regiella insecticola* infections where the symbiont was removed with antibiotics in strains 319 (empty squares) and 126 (solid squares), and artificial injections of *R. insecticola* strains 319 (empty triangles) and 313 (solid triangles), *Spiroplasma* strains 227 (empty circles) and 237 (solid dots), *Serratia symbiotica* strain 619 (empty diamonds) and *Rickettsiella* strain 620 (solid diamonds). Spearman's rank correlation tests for these relationships were never significant as shown in Supplementary Table 1

secondary symbionts. No significant differences in plant attractiveness to parasitoids were seen with the natural association but in the experiments with introduced *Regiella* there was a preference for plants that had been fed upon by aphids without the symbiont (Fig. 5, columns i–iv). We injected two *Spiroplasma* isolates into secondary symbiont-free aphids and found that wasps showed a significant preference for plants previously attacked by aphids without this symbiont (Fig. 5, columns v–vi). In an experiment with a single naturally occurring isolate of *Serratia symbiotica*, wasps showed a significant preference for plants that had been fed on by aphids without the symbiont (Fig. 5, column vii). Finally, in an experiment with a single-injected isolate of *Rickettsiella* sp. wasps were also attracted to plants fed on by aphids without secondary symbionts (Fig. 5, column viii). There was no correlation between aphid vigour (measured by relative progeny production) and wasp preference suggesting that the results are not affected by any influence of the symbiont on the damage caused by the aphid to the plant (Fig. 2b, Supplementary Table 1).

Discussion

Our study shows that plants infested with aphids carrying the symbiont *H. defensa* were less attractive to the parasitic wasp *A. ervi* through changes in herbivore-induced plant volatiles. We demonstrate this in a two-chamber olfactometer with a set of different *H. defensa* strains and aphid clones, and for three different *A. pisum* host plants. Reduced wasp attraction was not due to any chemical residue from the aphids remaining on the leaf, and the symbiont interfered with parasitic wasp recruitment through aphid-induced plant volatiles that were emitted systemically. In a population cage experiment, we demonstrate that changes in wasp attraction translate into reduced parasitoid attacks and hence increased fitness when aphids carried the symbiont. We therefore provide evidence of a previously unknown mechanism through which symbionts protect their aphid hosts from parasitic wasps.

Many strains of *H. defensa* provide protection from parasitic wasp attack[19, 20] but even if the host survives, its fitness is reduced relative to unparasitised individuals[30]. By undermining the ability of the host plant to recruit parasitic wasps the symbiont provides an added level of protection that may result in the avoidance of parasitic wasp attack. Our results can be explained by the symbiont disrupting the blend of herbivore-induced plant volatiles produced by the host plant so that it no longer signals the presence of a host to the parasitic wasp. Alternatively, the modified blend might signal the presence of a well-defended host, which is uneconomical for the wasp to attack in terms of potentially wasted eggs or wasted time. This latter explanation, however, is less likely because we found that non-protective symbiont species as well as non-protective *H. defensa* strains also caused the plant to be less attractive to wasps. We have also found that total volatile emissions were significantly reduced by the presence of *H. defensa*. A possible interpretation of this result is that aphid symbionts reduce parasitic wasp recruitment by suppressing signalling pathways downstream of the production of multiple volatile compounds. This may be particularly efficient in avoiding attacks by generalist natural enemies like polyphagous aphid predators, which relative to specialist enemies often use more general cues to locate their hosts[31]. It would be interesting to carry out experiments exposing treated plants to a complex community of natural enemies in the field to explore this question further.

Many plants respond to herbivory by the emission of specific mixtures of volatiles that attract natural enemies[10]. Plant defences are often triggered by specific elicitors in herbivore oral secretions[32]. Insects, however, have evolved strategies to overcome

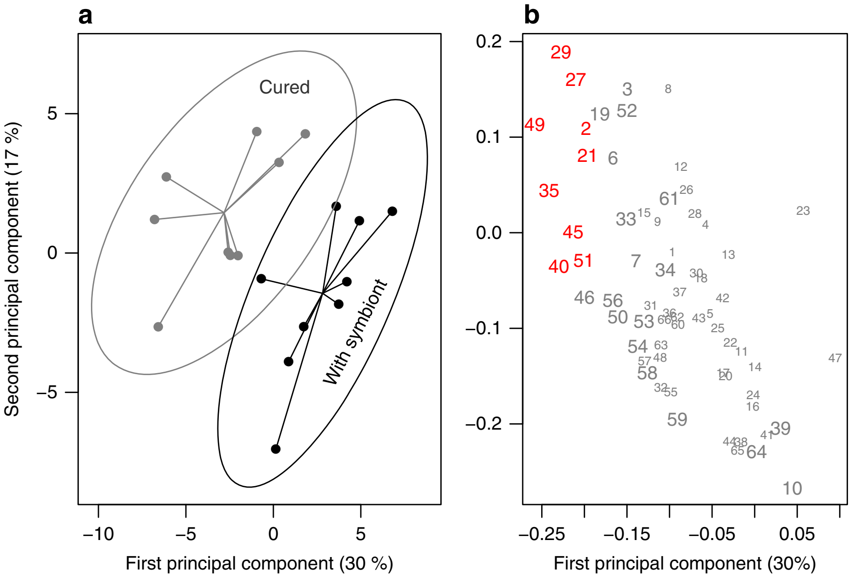

Fig. 3 Discriminant analysis of the volatiles collected in the plant headspace. Volatiles were collected from *Vicia faba* plants infested with *Acyrthosiphon pisum* aphids (strain 101) with or without the symbiont *Hamiltonella defensa* (antibiotic curing). **a** First two principal components of a PLS-DA plot. Each data point represents a plant, the centre of the star is the multivariate centroid, and the circle the 95% confidence interval. **b** PLS-DA loading plot with all compounds depicted with respect to the first two principal components. Compounds depicted in a larger font have a variable importance in projection (VIP) score larger than 1, and those in red are emitted in a significantly larger amount by plants induced with symbiont-free aphids (Supplementary Table 2). In this figure some overlapping points were slightly displaced to increase clarity

these defences using salivary effectors[33–36] or by mimicking plant hormones[37]. Although plants infested with aphids carrying the symbiont or without it emitted the same volatile compounds, we found quantitative differences in the volatile blends. More specifically, the quantities of nine volatile compounds were significantly lower from plants in the symbiont treatment. Among these, β-cubebene and α-amorphen have previously been shown to be emitted by plants infested by aphids, but not by healthy plants[15], which makes these two compounds potential attractants of the specialist wasp *A. ervi*. Studies combining gas-chromatography, electro-antennography and behavioural assays may help to identify the characteristics of blend components that influence the behaviour of the parasitic wasp. Further research is also needed to understand the molecular mechanisms through which symbionts in the aphid are able to affect plant physiology, possibly through changes in the phytohormone levels.

Products derived from the obligate symbiont *Buchnera aphidicola* have been found in the saliva of *A. pisum*, and are known to induce plant defences[38]. Plant defensive responses mostly depend on the pathways regulated by phytohormones and studies with whiteflies and the Colorado potato beetle have demonstrated that symbionts can downregulate levels of the phytohormone jasmonic acid in ways that benefit their hosts[5, 6]. It would be interesting to investigate if compounds derived from aphid facultative symbionts are present in the saliva and injected into the plant. The complete genome of *H. defensa* is available and it contains sequences similar to those coding for effector proteins in plant pathogens that have been implicated in plant recognition of bacterial pathogens, which can potentially play a role in manipulating plant phytohormonal responses[39]. As reviewed by Pineda et al.[40], the microbial symbionts of plants may also modulate the production of herbivore-induced plant volatiles by altering plant defensive responses, which underlies the importance of microbial influences on plant-insect interactions.

Most of the secondary symbionts of pea aphids provide some conditional fitness benefits for their hosts, chiefly in combatting abiotic or biotic challenges. Early work suggested that different symbionts had specific functions, *H. defensa* and *R. insecticola* in defence against parasitic wasps and fungal pathogens, respectively,[20, 25], and *S. symbiotica* against heat shocks[28]. More recent research has revealed a more complicated picture with some

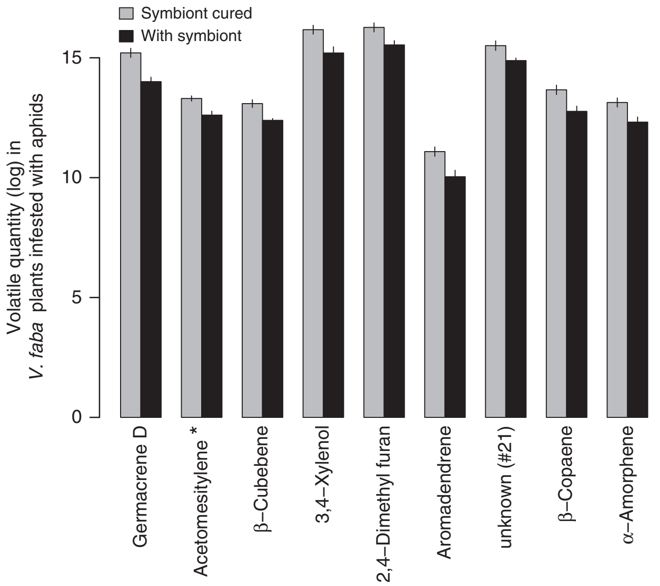

Fig. 4 Quantification of selected volatile compounds found in the plant headspace. Volatiles were collected from *Vicia faba* plants infested with *Acyrthosiphon pisum* aphids carrying the symbiont *Hamiltonella defensa*, or not. Values are expressed as peak areas divided by dry plant weight (g) and the bars show standard errors. As shown in Supplementary Table 2, pairwise comparisons were performed for those compounds with a 'variable importance in projection' (VIP) score larger than one in the Principal least squares discriminant analysis (PLS-DA). Only compounds whose quantity was significantly different after correcting *p*-values with the false discovery rate approach are included. *Acetomesitylene: 1,3,5-Trimethyl-2-acetylbenzene

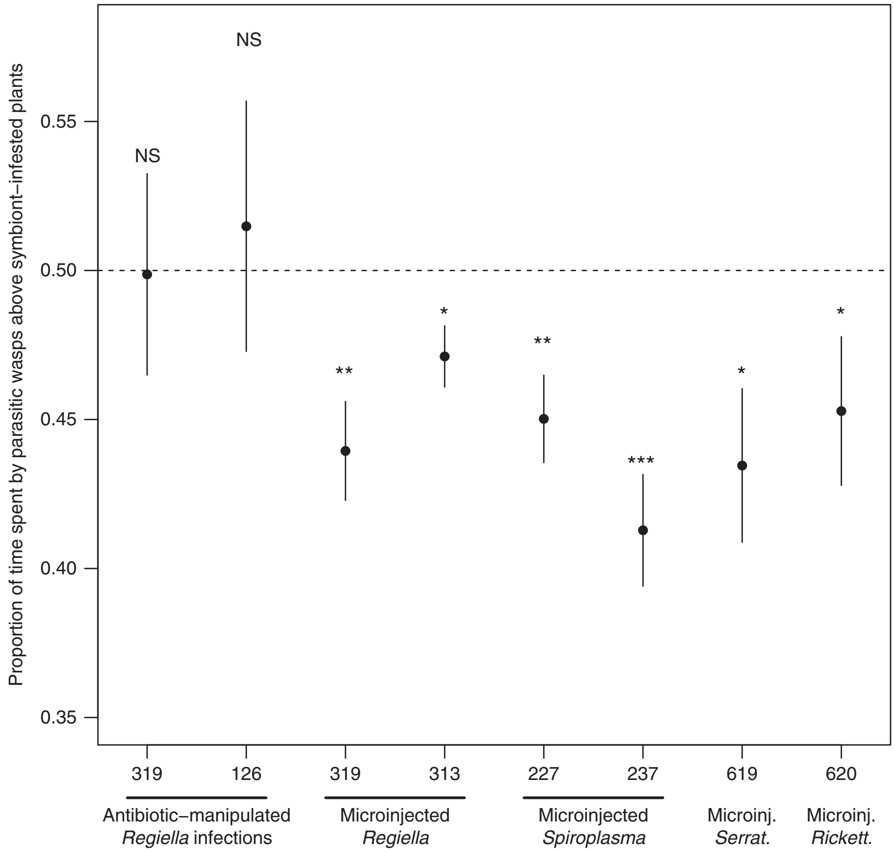

Fig. 5 Parasitic wasp preference for volatiles from plants infested with aphids with or without different endosymbiont species. Parasitic wasp (*Aphidius ervi*) response for volatiles emitted by *Vicia faba* plants infested with aphids (*Acyrthosiphon pisum*) with or without different symbiont species. For each symbiont strain tested, the bars show standard errors and the asterisks the significance of the deviation from no choice (t-test: NS: non-significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). *Regiella insecticola* ($n = 10$; column i—cured 319: $t_9 = -0.04$, $p = 0.4851$; ii—cured 126: $t_9 = 0.35$, $p = 0.6340$; iii—microinjected 319: $t_9 = -3.65$, $p = 0.0026$; iv—microinjected 313: $t_9 = -2.81$, $p = 0.0102$), *Spiroplasma* ($n = 10$; column v—microinjected 227: $t_9 = -3.40$, $p = 0.0039$; vi—microinjected 237: $t_9 = -4.65$, $p = 0.0006$), *Serratia symbiotica* ($n = 10$; column vii—microinjected 619: $t_9 = -2.54$, $p = 0.0158$) and *Rickettsiella* ($n = 10$; column viii—microinjected 620: $t_9 = -1.89$, $p = 0.0456$). Natural symbiont infections were removed with antibiotics in the *Regiella* strains 319 and 126, while the others were artificially microinjected into an aphid clone naturally lacking any secondary symbiont, and which was collected on *Lathyrus pratensis*

Table 1 Symbiont strains used, and the aphid *Acyrthosiphon pisum* biotype from which they were eliminated with antibiotics, or obtained when microinjected

Symbiont species	Symbiont strain	Aphid biotype	Experiment
Hamiltonella defensa	101	*Ononis spinosa*	1, 2, 3
H. defensa	132	*Lotus pedunculatus*	1
H. defensa	302	*Medicago sativa*	1
H. defensa	404	*L. pedunculatus*	1
Regiella insecticola	319	*Trifolium pratense*	4
R. insecticola	126	*T. pratense*	4
R. insecticola	313	*T. pratense*	4
Spiroplasma	227	*M. sativa*	4
Spiroplasma	237	*M. sativa*	4
Serratia symbiotica	619	*Lathyrus odoratus*	4
Rickettsiella	620	*Pisum sativum*	4
None (recipient)	NA	*Lathyrus pratensis*	1, 4

Information is given on the use of the different strains in each experiment. Microinjected symbionts were always injected into the same *A. pisum* lineage, which was obtained from *Lathyrus pratensis* plants

strains of a species failing to confer the expected advantage and in the case of *H. defensa* different isolates protecting against different wasp species[19]. In addition, new symbiont associations have been discovered with phenotypic effects that overlap with those previously investigated: different strains of *Spiroplasma* sp. associated with the pea aphid have particularly variable effects on its host[27]. It is thus not too surprising that the four additional symbionts we tested also influenced parasitic wasp recruitment. In the case of *R. insecticola*, the variation in response found is also not surprising because symbiont effects may depend on the symbiont strain, as well as on its interaction with the genotype of the aphid[30]. Further work on the mechanisms through which symbiont presence can affect the emission of plant volatiles may reveal the degree to which this phenomenon is a specifically selected adaptation or a byproduct of other processes through which the symbiont influences plant physiology, and whether the different symbionts influence volatile emission through a single or multiple mechanisms.

In conclusion, we show that microbial symbionts add another level of complexity to the already intricate role of plant volatiles in mediating the relationship between plants, herbivores and their natural enemies. We also demonstrate that they protect the host (and themselves) from parasitoid wasp attack both directly, by reducing the chances of successful wasp development, and indirectly, by reducing the probability of wasp attack. Aphids include some of the most important pests of temperate crops, and understanding these relationships may assist in the challenge of designing more environmentally benign pest control strategies. These strategies may include assessing the prevalence of protective symbionts in pest populations[41], and selection of plant varieties that once attacked by aphids, maximise the attraction to aphid natural enemies[42].

Methods

Experimental organisms. All aphids used in this study (Table 1) were collected from different host plant species in Oxfordshire (southern England) and maintained in the laboratory on broad bean plants (*V. faba*, cv. the Sutton) at constant conditions of 20 ± 1 °C and 70 ± 5% relative humidity with a 16:8 h light:dark (L:D) regime, to assure continuous asexual reproduction. After collection, aphids were screened using diagnostic PCRs for the eight common facultative endosymbionts known from this aphid species[43–45]. Symbionts were removed from naturally infected clones using a cocktail of antibiotics (ampicillin, cefotaxime and gentomicin) administered through the host plant. These antibiotics do not harm the obligate primary symbiont *Buchnera aphidicola*. New symbiont infections were created by microinjecting haemolymph from a donor aphid into a receiver clone which carried no natural facultative symbionts. All experiments were performed at least 10 aphid generations after manipulation. Before the experiments symbiont composition was checked using diagnostic PCRs. Further details of the experimental procedures can be found in refs. [18, 46,]], while a summary of the aphids and symbionts used in this study is provided in Table 1.

Aphidius ervi parasitic wasps were obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands) and maintained on an *A. pisum* clone that is naturally free from any facultative symbiont and which was not used in any of the behavioural experiments. Wasps used in the experiments were 2- to 3-day-old mated females, which for the 24 h prior to the experiment had been provided with diluted honey (10% solution) and aphids so that they could gain oviposition experience as in ref. [47]. The hosts were withdrawn two h before the experiment.

Effect of symbiont *H. defensa* on parasitic wasp attraction. The effect of *A. pisum* symbionts on the production of herbivore-induced plant volatiles and wasp preference was tested in a two-chamber olfactometer[48]. This olfactometer consists of a Perspex cylinder, which is divided longitudinally into two identical compartments. The cylinder is placed in a vertical position and the top end is closed off with thin mesh. A test plant is placed in each compartment, and parasitic wasps are released in an enclosed space above the mesh. Wasp preference for either of the two plants is assessed as the proportion of time wasps spent on top of each chamber. To trigger the induction of herbivore-induced volatiles by *V. faba* plants, a single plant was planted in a 1.1 L pot. Then, at the 2–3 leaf developmental stage (2-week-old plants), two clip cages containing 10 wingless adult female aphids each were placed on the two halves of the bottommost leaf. Aphids were allowed to feed on the plant for 5 days, and then removed. Every other day, aphid offspring was removed from the clip cage and counted as a measure of aphid vigour and feeding intensity. The protocol was slightly different for experiments with the plants *Ononis spinosa* and *Lotus pedunculatus*. Instead of using clip cages, 20 wingless adult females were placed onto a 6-week-old plant planted in a 1.1 litre pot. Plants were individually covered with a micro-perforated plastic bag to prevent aphids from escaping and the insects allowed to feed for 5 days before they were removed from the plant.

All tests were performed by comparing the attractiveness of plants which had been fed upon by genetically identical, clonal aphids, which differed only in their symbiont status. To prevent volatiles from the soil interfering with the behaviour of the parasitic wasp, pots were covered with aluminium foil. Five minutes after placing the plants in the olfactometer, a single parasitic wasp was released in the centre of the arena and after a minute's pause its behaviour was recorded for the following 6 min. Wasps that did not forage during the 4 min following their release were discarded. In each bioassay testing a specific symbiont strain or plant, the response of five female wasps was monitored. The mean response of these five wasps was considered a replicate, and this was repeated 10 times, each time with a new set of plants and wasps. The relative position of plants with symbiont-carrying and symbiont-free aphids was changed after every third replicate when the olfactometer was left open to allow any volatiles to disperse. The person recording the behaviour of the wasp was unaware of the treatment allocation to the two chambers. The effectiveness of the two-chamber olfactometer used here was assessed in preliminary tests with a limited number of plant replicates ($n = 4$). These tests showed that relative to plants that carried an empty clip cage, plants carrying a clip cage with aphids were more attractive to wasps.

We first compared the attraction of plants that had been fed on by aphids that naturally carried *H. defensa* with the same clone from which the symbiont had been removed (strains 101, 132 and 302, Table 1). We then repeated the experiment twice comparing an aphid clone that when collected from the field carried no facultative symbionts with the same clone into which one of two *H. defensa* isolates had been introduced by microinjection (strains 132 and 404, Table 1). With the exception of strain 101, these symbiont strains are known to confer direct protection against *A. ervi* in the laboratory[19]. To investigate if volatiles are produced systemically, one of the experiments with an introduced *H. defensa* isolate (strain 132) was repeated but with the leaf on which the aphids had fed covered with aluminium foil (Supplementary Fig. 1). Carrying symbionts may be costly and so reduce aphid vigour, and hence possibly cause less feeding damage to the plant and lower volatile emission. To control for this we repeated an experiment with the strains 132 and 101 (Table 1) but with double (20) the number of symbiont-bearing compared to symbiont-free aphids. Finally, we repeated two of the experiments with natural *H. defensa* infections but with the aphid feeding on the host plant species from which they were collected [*Ononis spinosa* (strain 101) and *Lotus pedunculatus* (strain 132)]. The pea aphid taxon is composed of host-adapted races or biotypes and microsatellite analyses had shown that these clones belonged to the biotypes associated with the two host plants[24].

Effect of symbiont *H. defensa* on parasitic wasp attack. To test whether differences in parasitoid recruitment translate into differences in parasitism, we compared the attack rates experienced by secondary symbiont-free aphids (sentinel aphids) feeding on plants that had previously been fed on by aphids with or without *H. defensa* (strain 101, 15 replicates per aphid type). The experiments were carried out in cubic gauze cages of 47.5 × 47.5 × 47.5 cm (BugDorm 44545 F, Taichung, Taiwan), which are arenas large enough for parasitoid wasps to show typical searching and oviposition behaviours[47]. Plants were prepared by allowing aphids of either of the two types to feed on them for 5 days before they were removed. Then, 30 genetically identical "sentinel" aphids were placed on each plant. When attacked by *A. ervi*, pea aphids drop from the plant and disperse within the cage. To identify which treatment they came from, each plant in the same cage received a different coloured aphid clone: one red and the other green. To control for any wasp preference, clone colour was stratified across treatments. The aphids were 3 days old when used and highly susceptible to parasitoid attack. A plant of each type was placed at opposite corners of the cage and 1 h later two mated female *A. ervi* wasps with oviposition experience were released in the centre of the cage. Wasps were allowed to search for and attack aphids for 2 h before being removed. The aphids were then collected and reared at 18 °C in Petri dishes on healthy leaves of *V. faba* with their petioles inserted into 2% agar gel to keep them fresh. Ten days later the number of parasitic wasp mummies and adult aphids obtained from each plant was counted.

Effect of symbiont *H. defensa* on volatile composition. We explored the effect of the symbiont *H. defensa* (strain 101) on the composition of volatiles in the headspace of *V. faba* plants previously infested with *A. pisum* aphids. Nine replicate plants that had been exposed to aphids with or without *H. defensa* were prepared as described above. Once aphids were removed, dynamic headspace sampling of volatiles was carried out in a climate chamber at 20 ± 1 °C. The soil and plant roots were carefully wrapped with aluminium foil to exclude any volatiles not coming from the plant. The plants were then individually placed into 2.5 l glass jars connected to an air flow. Humidified air, mixed with CO_2 at 400 p.p.m., was supplied to each jar. After 30 min, volatile collection started by drawing air out of the glass jar at a rate of 300 mL min^{-1} through a stainless steel tube filled with 200 mg Tenax TA filter (20/35 mesh; CAMSCO, Houston, TX, USA) for 4 h. Volatiles from a total of six glass jars were collected at the same time. Volatiles from test plants were collected in blocks of five and the sixth jar was kept as a control containing only a pot with soil wrapped in aluminium foil.

The volatiles were analysed as described in ref. [49]. A Thermo Trace Ultra Gas Chromatograph, in combination with a Thermo Trace DSQ quadrupole Mass Spectrometer (Thermo Fisher Scientific, Waltham, USA), were used for separation and detection of plant volatiles. Before analysis, moisture was removed from the Tenax adsorbent material by flushing with nitrogen (50 mL min^{-1}) for 10 min. The volatiles were then released from the Tenax filter using a helium flow of 20 mL min^{-1} for 10 min under an Ultra 50:50 thermal desorption unit (Markes, Llantrisant, UK) at 250 °C. At the same time, volatiles were re-collected in a universal solvent trap Unity (Markes) at 0 °C. The volatiles were then released and transferred to a ZB-5MSi analytical column [30 m × 0.25 mm I.D. × 0.25 μm F.T. with a 5 m built-in guard column (Phenomenex, Torrance, CA, USA)] by heating the solvent trap for an incremental 40 °C every second until a temperature of 280 °C was reached and then held for 10 min. The gas chromatograph oven was initially set at a temperature of 40 °C for 2 min before being raised by 6 °C a minute until it reached 280 °C at which it was kept for 4 min. The DSQ mass spectrometer operated in a scan mode with a mass range of 35–400 amu at 4.70 scans s^{-1} and the spectra were recorded in electron impact ionisation (EI) at 70 eV. The mass spectrometer transfer line and ion source were set at 275 and 250 °C, respectively.

Compounds were identified by comparing mass spectra data with those in the NIST 2005 and the Wageningen Mass Spectral Database of Natural Products MS libraries. Some compounds were also identified through linear retention indices based on the time they eluted from the gas chromatograph column relative to standard compounds. A target (single) ion for each compound was used for the measurement of peak area. Volatile samples from control jars (i.e., with just the pot and the soil wrapped in aluminium foil) were considered as blank samples. Volatiles recorded in these samples were thus treated as non-plant-related artefacts and subsequently excluded from the dataset.

Effect of other symbionts on parasitic wasp recruitment. The response of wasps to plants previously attacked by aphids carrying these different symbionts was assessed as in Experiment 1 with details of the aphid-symbiont strains involved given in Table 1. As in previous experiments, for *Regiella* strains 319 and 126 natural symbiont infections were removed with antibiotics, whereas the others were artificially microinjected into an aphid clone naturally lacking any secondary symbiont, and which was collected on *Lathyrus pratensis*.

Statistical analyses. All analyses were performed in R 3.3.1 (R development Core Team). Wasp preference in the two-chamber olfactometer followed a normal distribution and was analysed using *t*-tests with the null hypothesis being equal time allocation to the two treatments. The mean time allocation of the five wasps tested on a single plant pair served as the response variable in the analyses. Each test was repeated 10 times with new plants and new wasps. Spearman's rank correlation was used to test the relationship between the relative number of progeny produced by symbiont-bearing and symbiont-free aphids and wasp preference in the olfactometer. Differences in parasitism in the population cage experiment were explored with a paired *t*-test.

Plant volatile quantity (peak areas corrected by dry plant weight in grams) was log transformed before PLS-DA using the function plsda from the mixOmics package[50]. The significance of the treatment was assessed using a permutation analysis (9999 repetitions) implemented in the MVA.test from the RVAideMemoire package[51]. Variable importance in projection (VIP) scores calculated using PLSDA.VIP from the RVAideMemoire package were used to identify compounds important in treatment separation[52], which were then compared using *t*-tests after log transformation. In these comparisons *p*-values were corrected for multiple comparisons based on false discovery rates as implemented by the R function p.adjust. Relative to family-wise methods like Bonferroni, the false discovery rate method is less stringent in controlling type I errors, and is therefore more appropriate when a large number of comparisons is performed and some false positives are acceptable[53]. Since a different ion was used to quantify the various volatile compounds, total emissions cannot be obtained by summing up the amounts of the compounds obtained. Therefore, to test whether the symbiont had an overall effect on volatile emissions, a non-parametric two-sample sign test was used using the function SIGN.test from the BSDA package[54].

Data availability. The data sets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

R.G. and E.F. conceived and designed the research. E.F. wrote the Marie Curie project with input from H.C.J.G., R.G. and M.D. M.M. and C.Y. performed the behavioural experiments supervised by A.M., R.G. and E.F. B.T.W. collected, quantified and identified plant volatiles. A.M. created and characterised pea aphid lines. E.F. analysed data. H.C.J.G., R.G., M.D. and E.F. wrote the manuscript, and all authors contributed with revisions.

Additional information

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Supplementary Fig. 1
Image of a *Vicia faba* plant with its roots, soil and the leaf onto which clip cages were attached, covered with aluminum foil to test for systemic release of plant volatiles.

Supplementary Table 1

Spearman's rank correlation tests between the relative number of progeny produced by symbiont-carrying and symbiont-free *Acyrthosiphon pisum* aphids during five days on *Vicia faba* plants, and the proportion of time spent by parasitic wasps *Aphidius ervi* above symbiont-infested plants. Each row represents one test with a total of ten plant pairs (n=10). A plot showing these relationships is presented in Fig. 2.

Symbiont species	Symbiont strain	Cured / microinjected	Systemic response	S statistic	p-value	rho
Hamiltonella defensa	101	cured	no	212	0.4274	-0.28
H. defensa	132	cured	no	210	0.4483	-0.27
H. defensa	302	cured	no	170	0.9457	-0.03
H. defensa	132	microinjected	no	204	0.5139	-0.24
H. defensa	404	microinjected	no	156	0.8916	0.05
H. defensa	132	microinjected	yes	162	0.9728	0.02
Regiella insecticola	319	cured	no	142	0.6436	-0.18
R. insecticola	126	cured	no	172	0.9186	-0.04
R. insecticola	319	microinjected	no	186	0.7329	-0.13
R. insecticola	313	microinjected	no	174	0.8916	-0.05
Spiroplasma	227	microinjected	no	154	0.8648	0.07
Spiroplasma	237	microinjected	no	214	0.4070	-0.30
Serratia symbiotica	619	microinjected	no	264	0.0731	-0.60
Rickettsiella	620	microinjected	no	130	0.5599	0.21

Supplementary Table 2

Volatile compounds found in the headspace of *Vicia faba* plants infested with *Acyrthosiphon pisum* aphids carrying the symbiont *Hamiltonella defensa,* or not. Volatiles are ranked based on their variable importance in projection (VIP) in the Principal least squares discriminant analysis (PLS-DA). Volatile identity, type of volatile, its VIP score, retention time and its quantity are shown. Quantities are expressed as peak areas divided by dry plant weight (g). For the compounds with a VIP score larger than 1, pairwise comparisons on volatile quantity between plants carrying the symbiont or not (after log transformation) are also shown (n=9). P-values are corrected with the false discovery rate approach, and significant values are presented in bold. Mean (± SE) quantity of those compounds with a significant difference are represented in Fig. 4. Acetomesitylene: 1,3,5-Trimethyl-2-acetylbenzene, (E)-DMNT: (E)-4,8-Dimethylnona-1,3,7-triene, (E,E)-TMTT: (E,E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene, ar: Aromatic.

Supplementary Table 2 (cont.)

Compound	Id	Class	Ret. time (min)	VIP score	log (quantity) in		p-value
					Symbiont present	Symbiont removed	
Germacrene D	49	Terpenoid	21.43	1.6691	14±0.18	15.2±0.19	**0.0084**
Acetomesitylene	29	ar-Ketone	17.53	1.5813	12.61±0.16	13.3±0.11	**0.0234**
Nerolidol	52	Terpenoid	21.94	1.5484	7.02±1.78	10.95±0.26	0.0758
β-Cubebene	35	Terpenoid	19.47	1.5140	12.39±0.08	13.09±0.16	**0.0148**
3,4-Xylenol	27	Phenol	14.51	1.4906	15.2±0.26	16.17±0.19	**0.0304**
2,4-Dimethyl furan	2	Ether	3.72	1.3694	15.54±0.18	16.27±0.19	**0.0371**
Aromadendrene	45	Terpenoid	21.02	1.3560	10.04±0.27	11.09±0.19	**0.0304**
Unknown	21	NA	12.95	1.3455	14.88±0.1	15.51±0.2	**0.0371**
β-Copaene	40	Terpenoid	20.33	1.2981	12.77±0.22	13.66±0.2	**0.0304**
α-Amorphene	51	Terpenoid	21.80	1.2720	12.32±0.21	13.13±0.19	**0.0371**
2-Acetyl-3,5-dimethylfuran	19	Ketone	12.42	1.2445	14.91±0.23	15.59±0.16	0.0586
2,4-Pentanedione	3	Ketone	4.84	1.1952	15.77±0.5	16.84±0.25	0.1002
β-Myrcene	10	Terpenoid	9.82	1.1512	14.7±0.16	14.6±0.1	0.6406
Isogermacrene D	46	Terpenoid	21.07	1.1321	12.97±0.22	13.65±0.19	0.0755
(*E,E*)-2,4-Hexadienal	6	Aldehyde	6.98	1.1214	12.27±0.33	13.18±0.25	0.0758
3-Heptanone	7	Ketone	7.17	1.1089	12.07±0.14	12.54±0.22	0.1221
Unknown	33	NA	19.23	1.1087	11.19±0.35	12.3±0.45	0.1002
α-Cadinene	56	Terpenoid	22.57	1.0895	10.84±0.31	11.67±0.22	0.0758
Unknown sesquiterpene	50	Terpenoid	21.67	1.0756	12.36±0.22	13±0.21	0.0824
γ-Cardinene	53	Terpenoid	22.09	1.0278	12.48±0.26	13.14±0.23	0.1002
Methyl dihydrojasmonate	61	Ester	24.74	1.0140	12.69±0.4	13.29±0.29	0.2786
β-Atlantol	59	Terpenoid	23.79	1.0123	12.39±0.31	12.9±0.35	0.3177
Diethyl adipate	34	Ester	19.28	1.0009	9.7±0.54	10.88±0.75	0.2647
α-Caryophyllene	44	Terpenoid	20.86	1.0002	15.26±0.15	15.33±0.14	0.7372
δ-Cadinene	54	Terpenoid	22.26	0.9984	12.94±0.22	13.44±0.19	NA
(*E,E*)-TMTT	58	Terpenoid	23.34	0.9981	17.46±0.15	17.84±0.19	NA
Unknown	64	NA	30.01	0.9947	13.07±0.3	13.06±0.25	NA
β-Caryophyllene	39	Terpenoid	20.14	0.9817	16.12±0.16	16.05±0.14	NA
(*E*)-α-Bergamotene	41	Terpenoid	20.41	0.9810	14.02±0.15	13.97±0.24	NA
2,2,6-Trimethylcyclohexanone	15	Ketone	10.93	0.9796	13.18±0.12	13.64±0.21	NA
Chiloscyphone	57	Terpenoid	22.67	0.9793	13.54±0.21	14.12±0.29	NA
(*Z*)-α-Bergamotene	38	Terpenoid	20.01	0.9776	12.54±0.16	12.55±0.19	NA
3,3-Dimethyl-2,4-pentanedion	8	Ketone	7.82	0.9695	12.14±1.53	14.08±0.39	NA

Supplementary Table 2 (cont.)

Compound	Id	Class	Ret. time (min)	VIP score	log (quantity) in		p-value
					Symbiont present	Symbiont removed	
Geranyllinalool	65	Terpenoid	30.46	0.9667	13.12±0.26	13.09±0.38	NA
α-Copaene	32	Terpenoid	19.17	0.9449	12.76±0.1	12.97±0.11	NA
β-Springen	63	Terpenoid	29.36	0.9408	13.51±0.14	13.96±0.23	NA
(*E*)-Cadina-1,4-diene	55	Terpenoid	22.46	0.9266	11.36±0.16	11.64±0.17	NA
γ-Muurolene	48	Terpenoid	21.32	0.9094	12.1±0.22	12.54±0.23	NA
Clovene	31	Terpenoid	18.82	0.9052	11.14±0.27	11.66±0.21	NA
4-Isopropylcyclohexanol	23	Alcohol	13.24	0.8891	11.96±1.51	10.29±1.97	NA
Linalool	20	Terpenoid	12.61	0.8778	13.55±0.3	13.72±0.31	NA
(*Z*)-3-Hexen-1-ol, acetate	12	Ester	10.25	0.8555	17.95±0.18	18.17±0.11	NA
β-Funebrene	36	Terpenoid	19.61	0.8416	9.14±1.19	10.92±0.32	NA
(*Z*)-β-Ocimene	16	Terpenoid	11.03	0.8370	15.44±0.16	15.44±0.13	NA
(*E*)-Cadina-1(6),4-diene	47	Terpenoid	21.25	0.8276	9.19±0.27	7.52±1.44	NA
Alloocimene	24	Terpenoid	13.32	0.8256	13.29±0.19	13.3±0.16	NA
(*E*)-β-Ocimene	17	Terpenoid	11.35	0.8062	18.22±0.12	18.28±0.1	NA
Geranylgeraniol	66	Terpenoid	30.50	0.8046	14.03±0.13	14.38±0.23	NA
(*Z*)-3-Hexen-1-ol	5	Alcohol	6.47	0.8044	16.65±0.19	16.79±0.13	NA
Limonene	14	Terpenoid	10.79	0.7775	14.7±0.15	14.7±0.13	NA
(*E*)-DMNT	22	Terpenoid	13.00	0.7410	16.14±0.21	16.25±0.24	NA
(*E*)-β-Ocimene epoxide	26	Terpenoid	13.65	0.7395	14.25±0.21	14.56±0.2	NA
Unknown	60	NA	24.33	0.7359	10.6±1.35	12.09±0.26	NA
(*E,E*)-Cosmene	25	Terpenoid	13.34	0.6993	14.5±0.19	14.61±0.11	NA
Anisole	9	ar-Ether	7.96	0.6942	13.69±0.28	14.15±0.17	NA
1,5,8-p-Menthatriene	18	Terpenoid	12.09	0.6938	12.71±0.2	12.88±0.11	NA
Unknown	11	NA	10.20	0.6857	12.87±0.18	12.91±0.1	NA
(*Z*)-Lanceol acetate	62	Ester	28.35	0.6684	14.15±0.11	14.47±0.27	NA
1-Butanol	1	Alcohol	3.10	0.6552	15.68±0.22	16.06±0.25	NA
(*Z*)-Jasmone	37	Ketone	19.63	0.6488	10.05±1.29	11.43±0.25	NA
α-Cubebene	30	Terpenoid	18.57	0.6145	9.53±1.22	10.52±0.12	NA
Methyl salicylate	28	ar-Ester	15.01	0.6035	15.65±0.25	15.96±0.29	NA
(*E*)-β-Farnesene	43	Terpenoid	20.82	0.6004	14.18±0.13	14.42±0.27	NA
(*E*)-2-Hexenal	4	Aldehyde	6.39	0.5831	12.7±0.26	12.94±0.25	NA
Sesquisabinene	42	Terpenoid	20.56	0.5243	11.87±0.17	12.01±0.27	NA
3-Methylanisole	13	ar-Ether	10.57	0.2948	13.15±0.22	13.25±0.17	NA

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LETTER

Defensive insect symbiont leads to cascading extinctions and community collapse

Dirk Sanders,[1] Rachel Kehoe,[1] FJ Frank van Veen,[1] Ailsa McLean,[2] H. Charles J. Godfray,[2] Marcel Dicke,[3] Rieta Gols[3] and Enric Frago[3]*

Abstract

Animals often engage in mutualistic associations with microorganisms that protect them from predation, parasitism or pathogen infection. Studies of these interactions in insects have mostly focussed on the direct effects of symbiont infection on natural enemies without studying community-wide effects. Here, we explore the effect of a defensive symbiont on population dynamics and species extinctions in an experimental community composed of three aphid species and their associated specialist parasitoids. We found that introducing a bacterial symbiont with a protective (but not a non-protective) phenotype into one aphid species led to it being able to escape from its natural enemy and increase in density. This changed the relative density of the three aphid species which resulted in the extinction of the two other parasitoid species. Our results show that defensive symbionts can cause extinction cascades in experimental communities and so may play a significant role in the stability of consumer-herbivore communities in the field.

Keywords

Acyrthosiphon pisum, Aphid, *Aphidius ervi*, cascading extinction, defensive symbiosis, endosymbiont, experimental community ecology, *Hamiltonella defensa*, indirect effect, parasitoid.

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INTRODUCTION

Virtually all animals are under strong natural selection to avoid predation, parasitism or pathogen infection, and as a consequence, they have evolved a variety of behavioural, mechanical, structural and chemical defences (Evans & Schmidt 1990; Eisner *et al.* 2007). It is increasingly becoming recognised that a further way animals can acquire protection against natural enemies is through association with microbial symbionts (Flórez *et al.* 2015). These defensive symbioses are particularly well studied in herbivorous insects (Hansen & Moran 2014; Oliver *et al.* 2014). Obligate insect microbial symbionts have long been known to be essential for some species because they provide essential nutrients absent in their diets (Barbosa *et al.* 1991; Douglas 2015). The last few decades have seen increasing interest in the evolution, diversity and persistence of facultative associations, and in particular, in those with a defensive role. Facultative defensive symbionts can provide their insect host with increased protection from predators, pathogens and parasitoids (reviewed in Flórez *et al.* 2015). Laboratory experiments with aphid and *Drosophila* populations have shown that the presence of natural enemies can lead to an increase in the frequency of defensive symbionts (Oliver *et al.* 2008; Jaenike & Brekke 2011). In natural populations, the benefits conferred by defensive symbionts can allow their insect hosts to spread spatially (Cockburn *et al.* 2013), and even within the same season, natural enemy pressure can rapidly increase the proportion of individuals carrying defensive microorganisms (Smith *et al.* 2015).

Research on defensive symbionts has tended to focus on their direct effects on the interaction between host and natural enemy (Oliver *et al.* 2010; Frago *et al.* 2012). However, recent advances in insect community ecology have made it increasingly clear that changes in direct interactions between a pair of species can have far-reaching indirect effects within networks of interacting species (Saterberg *et al.* 2013; Stam *et al.* 2013). Indirect interactions occur when one species affects the dynamics of a second not by a direct trophic or behavioural effect but mediated through the density, behaviour or trait of a third (or more) species. Indirect interactions can be important in promoting species persistence, community stability and ultimately maintaining higher levels of diversity (van Veen *et al.* 2005; Ives & Carpenter 2007; Estes *et al.* 2011). The elimination of indirect interactions can destabilise ecological communities and lead to extinction cascades (Sanders *et al.* 2013, 2015; Saterberg *et al.* 2013). To give an example, community persistence can be enhanced when multiple consumer species specialise on different, potentially competing prey (Vandermeer 1980; Sanders & van Veen 2012). If a particular consumer species is lost or becomes rare, interspecific competition between prey species can increase leading to their extinction, and as a consequence consumer species can also be lost, an extinction cascade (Sanders *et al.* 2013, 2015). Identifying indirect interactions, in this case between the focal

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[1] Centre for Ecology & Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn Campus, Penryn, Cornwall TR10 9FE, UK
[2] Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK
[3] Laboratory of Entomology, Wageningen University, P.O. Box 16, Wageningen 6700AA, The Netherlands
*Correspondence and present address: Enric Frago, CIRAD, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France. E-mail: enric.frago@gmail.com.

consumer, the prey it does not attack, and the other consumers, is the key to understanding community stability. Indirect interactions are likely to be particularly important in maintaining the diversity of insect herbivore communities because they typically support a complex of web of natural enemies, many of which are moderately to highly specialised (van Veen *et al*. 2006). The introduction of a defensive symbiont into a host population can act to remove a specific natural enemy from a community and we hypothesise that this can have effects not only on the host species but also on other members of the community mediated by indirect interactions.

The pea aphid (*Acyrthosiphon pisum*) is a model system for the study of insect symbiosis, and most individuals carry one or two species of facultative symbionts (Oliver *et al*. 2006, 2010; Henry *et al*. 2013). These include the endosymbiont *Hamiltonella defensa*, the first microbe found to have a protective effect against parasitic wasps (Oliver *et al*. 2003, 2005), though subsequent studies have shown that both defensive and non-defensive strains of this symbiont occur in *A. pisum* (Mclean & Godfray 2015). Clonal lines of asexually reproducing pea aphids can be established in the laboratory, and *H. defensa* can be removed using specific antibiotics or introduced by injection. Here, we study the effect of this symbiont on an aphid–parasitoid community composed of three aphid species (*Acyrthosiphon pisum*, *Aphis fabae* and *Megoura viciae*) and their associated specialist parasitic wasps (*Aphidius ervi*, *Lysiphlebus fabarum* and *Aphidius megourae* respectively). In this community (made up of a particular combination of genotypes), all species are required for long-term persistence in experimental cage populations (Sanders *et al*. 2013, 2015). We hypothesise that the introduction of a defensive symbiont will destabilise this community and trigger an extinction cascade, and test this with *H. defensa* in *A. pisum*. We established four different types of replicated experimental communities, identical apart from the *A. pisum* (Fig. 1). Two community types included an *A. pisum* clone that naturally hosted a protective form of *H. defensa*; in one community, the aphids were in their natural, infected state but in the other, the symbiont had been removed using antibiotics. The other two communities included a different clone of *A. pisum* which naturally hosted a non-protective form of the symbiont; in one community, the aphids retained their symbiont and in the other, the symbiont had been removed. We hypothesised that the protective endosymbiont will weaken the interaction between *A. pisum* and its associated parasitoid *A. ervi* leading to higher *A. pisum* densities. This would affect community stability through increased interspecific competition and a reduction in the densities of the other two aphid species which will increase the risk of their extinction or the extinction of their parasitoids. In the communities that included *A. pisum* with the non-protective symbiont strain, we did not expect *A. pisum* densities to increase or for there to be indirect effects influencing community stability. During the course of the study, we found that cured lineages of the aphid clone that carried the protective symbiont had higher population growth rates than the clone that carried the non-protective variant. This led us to predict that when comparing communities with the two different symbiont-free *A. pisum* clones, the lower competitive ability of the non-protective clone will lead to reduced densities of *A. pisum* and increased extinction of the parasitoid *A. ervi*. At the community level, the loss of *A. pisum* aphids and *A. ervi* parasitoids would also affect community stability through changes in interspecific competition. To understand better how non-host aphids might affect a parasitoid species' foraging behaviour and ultimately trigger its extinction, we conducted a behavioural experiment with the parasitoid *A. ervi*. We hypothesised that attacks on its own host, *A. pisum*, will be reduced in the presence of non-host aphids that altered parasitoid foraging.

MATERIAL AND METHODS

Study system

Replicated plant–aphid–parasitoid communities were constructed. They consisted of bean plants (*Vicia faba*, L., var. the Sutton) fed upon by three species of aphid: *Acyrthosiphon pisum* (Harris), *Aphis fabae* (Scopoli) and *Megoura viciae* (Buckton). Each aphid species was attacked by a specialist parasitoid species: *Aphidius ervi* (Haliday), *Lysiphlebus*

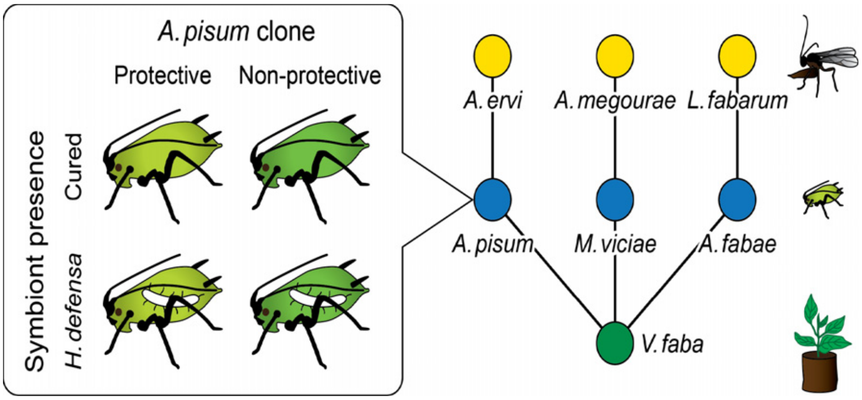

Figure 1 Experimental design. Cages were established with three species of aphids (*Acyrthosiphon pisum*, *Aphis fabae* and *Megoura viciae*) feeding on *Vicia faba*, along with their specialist parasitoids (*Aphidius ervi*, *Lysiphlebus fabarum* and *Aphidius megourae* respectively). The clone and symbiont infection status of *A. pisum* differed between cages: clones originally hosted either a protective symbiont or a non-protective symbiont, and were either in their natural, infected state or had previously been cured of *Hamiltonella defensa*.

fabarum (Marshall) and *Aphidius megourae* (Stary) respectively (Fig. 1). In the experiments, two clones of *A. pisum* in which we manipulated the presence of their natural secondary symbiont, *H. defensa*, were employed. Symbiont removal was achieved using a specific antibiotic curing protocol which does not affect the primary symbiont (McLean *et al.* 2011). The first clone was collected on *Medicago sativa* and the strain of *H. defensa* it carries confers strong resistance again the parasitoid *A. ervi*. The second clone was collected on *Ononis spinosa* and carried a strain of *H. defensa* that appears to provide no protection against *A. ervi* (Mclean & Godfray 2015). Below, we shall refer to these *H. defensa* strains as protective and non-protective. *H. defensa* was absent from the two other aphid species. The *Medicago* strain of *A. pisum* also carried a second facultative symbiont, X-type, which was not affected by the procedure we used to manipulate *H. defensa* presence. Note that while the two *A. pisum* clones belong to biotypes associated with *Medicago* and *Ononis*, they, like nearly all pea aphid biotypes, flourish on *Vicia faba* which has been described as a 'universal host' (Ferrari *et al.* 2008).

Experiment

The experiment consisted of four treatments with identical species present, but which varied in the clone and symbiont status of *A. pisum*. Two *A. pisum* clones (*Medicago* vs. *Ononis*) with the presence or absence of its natural strain of *H. defensa* were used. Communities were maintained in 47.5 cm^3 cube gauze cages (BugDorm 44545, Taichung, Taiwan). They were initiated by introducing five wingless adults of the three aphid species spread across four pots (15 cm diameter) each of which contained four 2-week-old bean plant seedlings. Two adult mated females of each parasitoid species were added 2 weeks later, and a second pair at week three. This ensured an overlap of parasitoid generations. Each treatment was replicated 10 times in a controlled temperature room at 20 ± 3 °C and a 16/8 h light–dark cycle. To ensure all treatments were exposed to similar conditions, the cages were arranged in ten spatial blocks each containing the four different community types. Beginning 2 weeks after the introduction of the parasitoids, the numbers of aphids and parasitoid mummies on half the plants in each cage was counted once a week. On some occasions, parasitoids were recorded but no hosts. This happened when aphids were very rare (prior to extinction) and by chance none occurred on the half of the plants that were sampled, and because of the natural lag between aphid and parasitoid extinction. Twice a week, the two oldest pots of bean seedlings were replaced by two containing fresh 2-week-old seedlings; the old stems from the discarded plants were retained in the cages to avoid loss of insects from the system. Our previous studies had shown that this protocol allows the long-term persistence of this community of competing aphid species and their natural enemies (Sanders & van Veen 2012; Sanders *et al.* 2013, 2015).

Behavioural experiment

We measured the impact of non-host aphids on parasitoid foraging behaviour in *A. ervi*. The upper part of a 3-week-old bean plant (including two leaves) was cut off, and the stem inserted upright in 10% agar to maintain freshness. In addition to 20 *A. pisum* aphids, the different treatments contained 20 *M. viciae* aphids, 20 *A. fabae* aphids, or 10 of each aphid species. All aphids were 3–4 days old, and plants with *A. pisum* only were considered as controls. The plants with aphids were placed in a 250-mL glass beaker, and after 20 min, a mated and experienced female wasp was released inside. The number of attacks on the different aphid species was recorded over a 10-min period. We considered a parasitoid attack when females exhibited the stereotypical egg laying behaviour in Braconidae aphid parasitoids which consists of extending the abdomen frontally through the legs, and touching the aphid with the abdomen's terminal part. Six wasps that attacked < 5 aphids were excluded from the analyses.

Statistical analysis

All data were analysed using the open source software R 3.1.3 (R development Core Team). We calculated the initial growth rate of the different aphid populations in the first 30 days of the experiment before the emergence of the first generation of parasitoids (growth rate = $(N(30) - N(0))/30$ where $N(x)$ is population density on day x). We used ANOVA to test the effects of clone and symbiont presence on initial population growth rate. The impact of these treatments on aphid and parasitoid population dynamics were analysed by building independent linear mixed effects models for each clonal lineage with symbiont presence as fixed factor. To account for systematic trends over time, week, and week squared were included as covariates while cage nested in block was included as a random factor. Because the residuals of the models showed significant temporal autocorrelation, a first-order autoregressive term was included. Model simplification was carried out by sequentially removing non-significant interactions within the function *lme* from the package *nlme* (Pinheiro *et al.* 2015). We additionally analysed aphid and parasitoid dynamics in all four treatments at the same time by building similar models, but including clonal lineage, symbiont and their interaction as fixed factors. Percent variance explained by fixed factors in mixed models was estimated as pseudo-R^2 values using the function *r.squaredGLMM* from the package *MuMIn* (Barton 2016). Persistence of the different species in the community was analysed with nonparametric Kaplan–Meier survival analysis using the function *survdiff* in the package *survival* (Harrington & Fleming 1982). Species that persisted in cages until the end of the experiment were treated as censored data.

Aphid relative abundance was analysed using generalised linear mixed models assuming a binomial error distribution and using the logit link function. The dependent variable was the bivariate variable containing 'abundance of aphid species *i*' and 'total aphid abundance – abundance of aphid species *i*', where '*i*' can be the abundance of *A. fabae*, *M. viciae*, or *A. pisum*. Symbiont or clone treatments were included as fixed factors, and week and week squared as covariates. Week squared was included in the models as a covariate to account for systematic nonlinear trends over time and to increase model fit. Block and replicate nested in block were treated as random factors and we also included a random slope for the week effect per replicate. Since a degree of over-dispersion was detected, an observation-level random factor was also included (Browne *et al.* 2005). The analysis used the function *glmer* from the package *lme4* (Bates *et al.* 2014). Model simplification was carried out by sequentially removing non-significant interactions. To obtain 95% credible intervals for the model predictions, we used Bayesian methods to draw a random sample of 1000 values from the posterior distribution of the model parameters. This was done employing the function *sim* from the package *arm* (Gelman & Yu-Sung 2015). From these 1000 model parameter sets, predicted values were calculated and their 2.5 and 97.5% quantiles were used as lower and upper limits of the 95% credible intervals. Parasitoid attacks in the behavioural experiment were analysed with generalised linear models assuming a quasi-poisson error distribution.

RESULTS

The effect of the protective symbiont on *A. pisum* and its parasitoid

We predicted that the presence of the protective symbiont would lead to higher *A. pisum* densities and negatively affect its parasitoid, *A. ervi*. The results supported this prediction. The mean cumulative density of *A. pisum* was 1.6 times greater in replicates with the symbiont compared to without ($F_{1,9} = 6.93$, $P = 0.027$), while the mean cumulative density of *A. ervi* was over 16 times less ($F_{1,9} = 98.32$, $P < 0.001$; Table S1; Fig. 2). The symbiont effect on *A. ervi* numbers varied through time as revealed by a significant interaction between time and treatment ($F_{1,197} = 5.89$, $P = 0.016$; Table S1; Fig. 2). In replicates with the symbiont, the parasitoids always had low densities, whereas without the symbiont, an initial peak was followed by a decrease in density after the third week ($F_{1,197} = 5.89$, $P = 0.016$; Table S1; Fig. 2). The relative abundance of *A. pisum* was slightly higher in those cages with the symbiont, although this was marginally non-significant ($Z = 1.89$, $P = 0.058$; Table S2; Fig. 4). *A. pisum* aphids became extinct in 10% of the cages irrespective of the presence of the symbiont ($\chi^2_2 < 0.01$, $P = 0.970$; Table S1; Fig. 5: panel A1). The parasitoid, however, went extinct in all cages with the symbiont present while it persisted in all communities without the symbiont, a significant difference ($\chi^2_2 = 22.00$, $P < 0.001$; Table S1; Fig. 5: panel P1). A detailed description of the statistical analyses is provided in the online supplementary material (Tables S1 and S2).

The effect of the protective symbiont at the community level

We expected that the higher densities of *A. pisum* brought about by the presence of the protective symbiont would, through greater resource competition, negatively affect the two other aphids, *M. viciae* or *A. fabae*, and that this would in turn reduce the densities and persistence of their specific parasitoids. The cumulative mean numbers of *M. viciae* and *A. fabae* did not differ between the two treatments (*M. viciae:* $F_{1,9} = 4.79$, $P = 0.057$; *A. fabae:* $F_{1,9} = 2.90$, $P = 0.123$; Table S1; Fig. 2). The effect of the symbiont on *M. viciae* numbers became stronger over time (there was a significant interaction between time and the symbiont treatment, $F_{1,197} = 11.11$, $P < 0.001$; Table S1; Fig. 2). Despite the absence of differences in total abundance, *M. viciae* relative abundance significantly declined from week six onwards, to < 1% in treatments with the symbiont compared to 5–7% without the symbiont ($Z = 3.43$, $P < 0.001$; Table S2; Fig. 4). Similarly, *A. fabae* relative abundance declined from 27 to 0.9% when the symbiont was present, a significant difference ($Z = -2.24$, $P = 0.025$; Table S2; Fig. 4). Towards the end of the experiment, *A. megourae* parasitoid numbers were lower when the symbiont was present (symbiont effect: $F_{1,9} = 5.22$, $P = 0.048$; symbiont × week effect: $F_{1,197} = 8.07$, $P = 0.005$; Table S1; Fig. 2). The presence of the symbiont did not affect the numbers of the parasitoid *L. fabarum* ($F_{1,9} = 2.26$, $P = 0.137$; Table S1; Fig. 2). There was an effect of the symbiont on the persistence of *M. viciae* ($\chi^2_2 = 7.09$, $P = 0.008$), but not of *A. fabae* ($\chi^2_2 = 3.45$, $P = 0.063$; Table S1; Fig. 5: panels A2 & A3). *M. viciae* became extinct in all cages when the symbiont was present, but only in 30% of replicates without the symbiont. The greatest indirect effect of the presence of the symbiont was on the persistence of the specific parasitoids *A. megourae* and *L. fabarum* which became extinct in all cages when the symbiont was present, but persisted in all communities when the symbiont was absent (*A. megourae* $\chi^2_2 = 21.40$, $P < 0.001$; *L. fabarum* $\chi^2_2 = 20.30$, $P < 0.001$; Table S1; Fig. 5: panels P2 & P3).

The effect of the non-protective symbiont on *A. pisum* and its parasitoid

We predicted that the presence of the non-protective symbiont would not lead to higher *A. pisum* aphid densities (and might even reduce their numbers if symbiont carriage was costly), and that it would have no effect on *A. ervi* dynamics. *A. pisum* densities were slightly lower in the presence of the symbiont, but overall there were no significant differences in *A. pisum* or *A. ervi* densities, and their interaction with time (*A. pisum:* $F_{1,9} = 3.51$, $P = 0.094$; *A. ervi:* $F_{1,9} = 0.20$, $P = 0.669$; Table S1; Fig. 3). The relative abundance of *A. pisum* was not affected by the symbiont, but the symbiont had a significant effect on the time course of relative *A. pisum* abundances (symbiont effect: $Z = 1.21$, $P = 0.226$; symbiont × week effect: $Z = -2.64$, $P = 0.008$; Table S2; Fig. 4). By the end of the experiment, *A. pisum* relative abundance was very low in both treatments, but during week four to eight, abundances were lower when the symbiont was present (Fig. 4). Aphid and parasitoid persistence were also unaffected by the symbiont (*A. pisum:* $\chi^2_2 = 2.37$, $P = 0.123$; *A. ervi:* $\chi^2_2 = 0.49$, $P = 0.486$; Table S1; Fig. 5: panels A4 & P4).

The effect of the non-protective symbiont at the community level

We did not expect to see any community consequences of the presence of the non-protective symbiont in *A. pisum* aphids. The presence of the symbiont did not affect *M. viciae* and *A. megourae* densities (*M. viciae:* $F_{1,9} = 1.68$, $P = 0.227$;

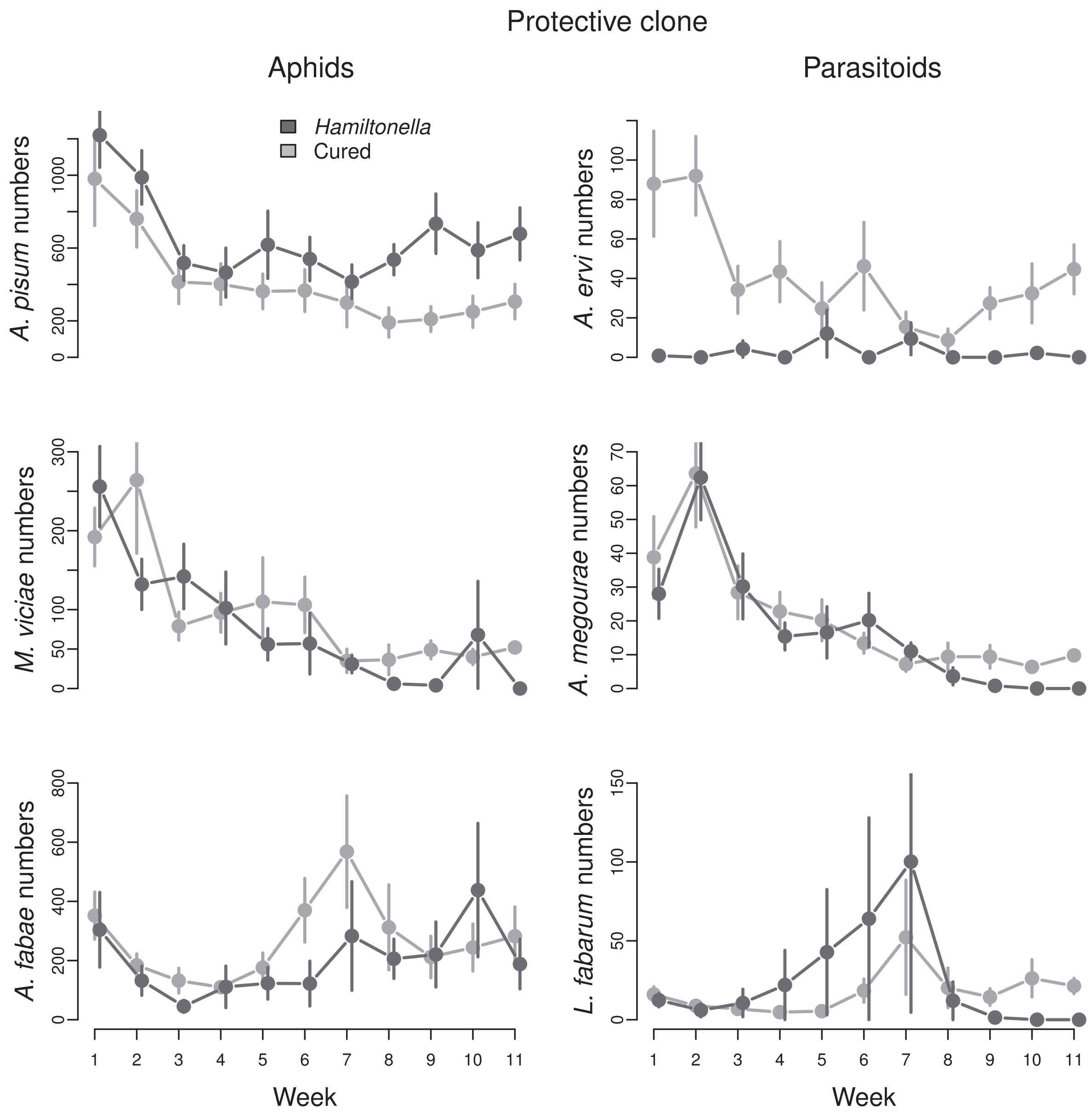

Figure 2 Long-term dynamics of the community where the symbiont status was manipulated in the *Acyrthosiphon pisum* aphid clone carrying the protective *Hamiltonella defensa* strain. Dark grey lines and bars represent species abundance (± SE) in replicates where the symbiont was present, and light grey lines and bars represent those where the symbiont was absent.

A. megourae: $F_{1,9} = 2.93$, $P = 0.121$; Table S1; Fig. 3) or their persistence (*M. viciae:* $\chi^2_2 = 0.16$, $P = 0.688$; *A. megourae:* $\chi^2_2 = 3.10$, $P = 0.078$; Table S1; Fig. 5: panels A5 and P5), but the relative abundance of *M. viciae* was lower when the symbiont was present, a marginally significant difference ($Z = -1.96$, $P = 0.049$; Table S2; Fig. 4). However, there was an effect of symbiont presence on the other aphid–parasitoid pair. *A. fabae* numbers were on average 1.9 times higher when the symbiont was present ($F_{1,9} = 6.62$, $P = 0.030$; Table S1; Fig. 3) and while this species became the dominant aphid in both treatments, this occurred more quickly in the symbiont replicates ($Z = 2.38$, $P = 0.017$; Table S2; Fig. 4). *A. fabae* became extinct in 50% of the cages when the symbiont was absent, but persisted in 90% of them when the symbiont was present, a marginally non-significant difference ($\chi^2_2 = 3.76$, $P = 0.053$; Table S1; Fig. 5: panel A6). Differences in the mean densities of the parasitoid *L. fabarum* were marginally significant ($F_{1,9} = 5.44$, $P = 0.045$; Table S1; Fig. 3). This parasitoid never went extinct in the presence of the symbiont, but it was lost in half the replicates where the symbiont was absent ($\chi^2_2 = 6.34$, $P = 0.019$; Table S1; Fig. 5: panel P6).

The effect of *A. pisum* clonal differences on *A. pisum* and its parasitoid

The two pea aphid clones used in the experiment were chosen because they were the natural hosts of the protective and non-protective symbionts strains, rather than to test any *a priori* hypotheses. During the establishment of our experimental

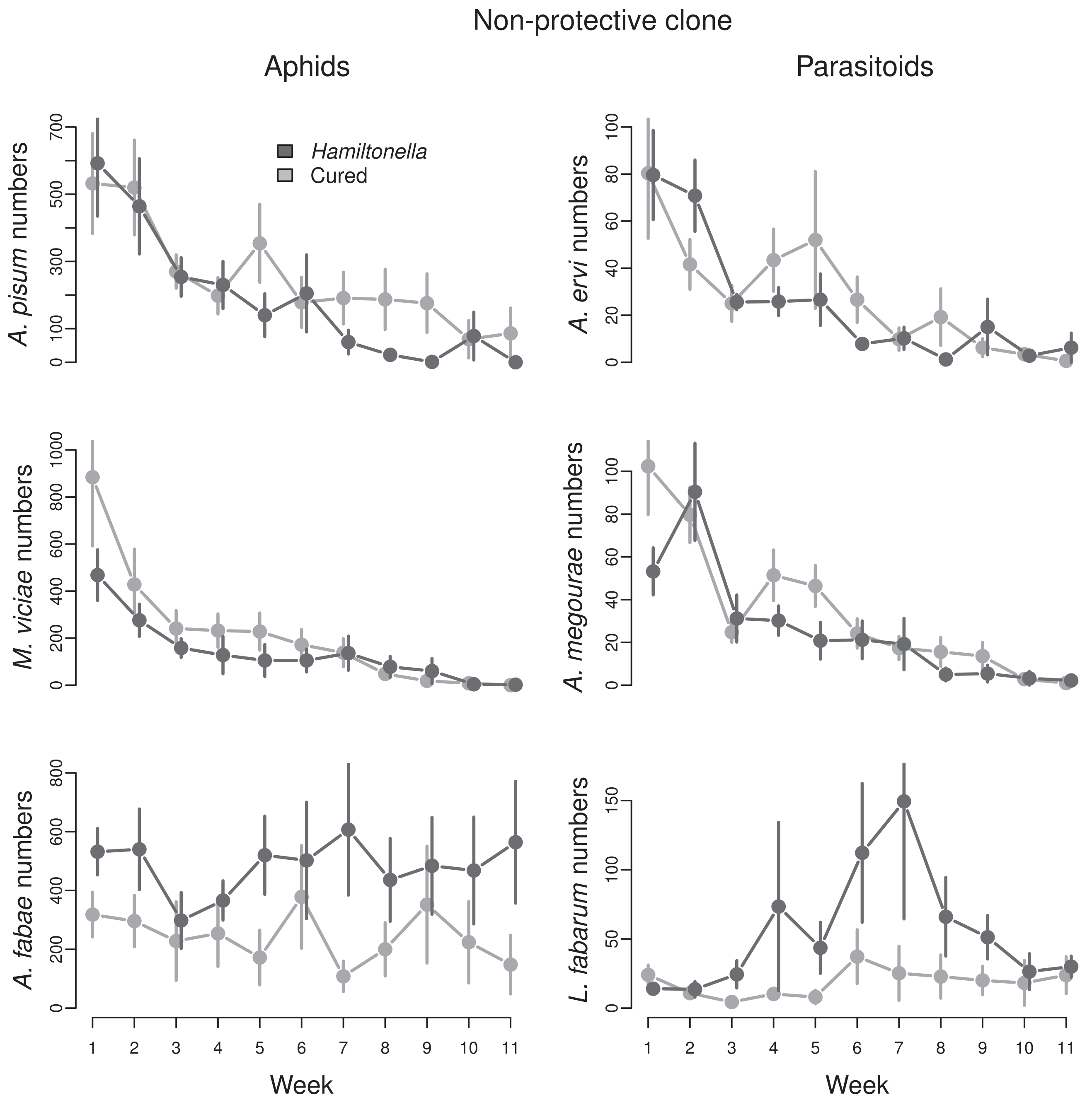

Figure 3 Long-term dynamics of the community where the symbiont status was manipulated in the *Acyrthosiphon pisum* aphid clone carrying the non-protective *Hamiltonella defensa* strain. Dark grey lines and bars represent species abundance (± SE) in replicates where the symbiont was present, and light grey lines and bars represent those where the symbiont was absent.

communities, it was noted that in the absence of the symbiont, the *A. pisum* clone that hosted the protective symbiont had a significantly higher initial population growth rate compared to the clone that carried the non-protective one (15.9 ± 2.3 (mean ± SE) vs. 8.0 ± 1.6; $F_{1,36} = 8.05$, $P = 0.007$). Although differences in growth rate were not affected by the presence of the symbiont (effects of symbiont: $F_{1,36} = 0.62$, $P = 0.434$; clone × symbiont interaction: $F_{1,36} = 0.23$, $P = 0.638$), we analysed insect dynamics only in the replicates without symbionts to avoid any possible confounding effects of the bacteria. The difference in initial population growth rate was reflected in significantly lower densities of *A. pisum* and *A. ervi* for the slower growing clone (*A. pisum*: $F_{1,9} = 8.09$, $P = 0.019$; *A. ervi*: $F_{1,9} = 6.23$, $P = 0.034$; Table S1; Figs. 3 and 4). Relative *A. pisum* abundance was also lower for the slower growing clone (27 vs. 44%, $Z = -2.38$, $P = 0.017$; Table S2; Fig. S4), and both *A. pisum* and its associated parasitoid had significantly greater probabilities of extinction (*A. pisum:* $\chi^2_2 = 6.33$, $P = 0.012$; *A. ervi:* $\chi^2_2 = 13.22$, $P < 0.001$; Table S1; Fig. 5: solid lines in panels A1, A4, P1 & P4). To assess the effect of the symbiont on the two different *A. pisum* clones, we also analysed aphid

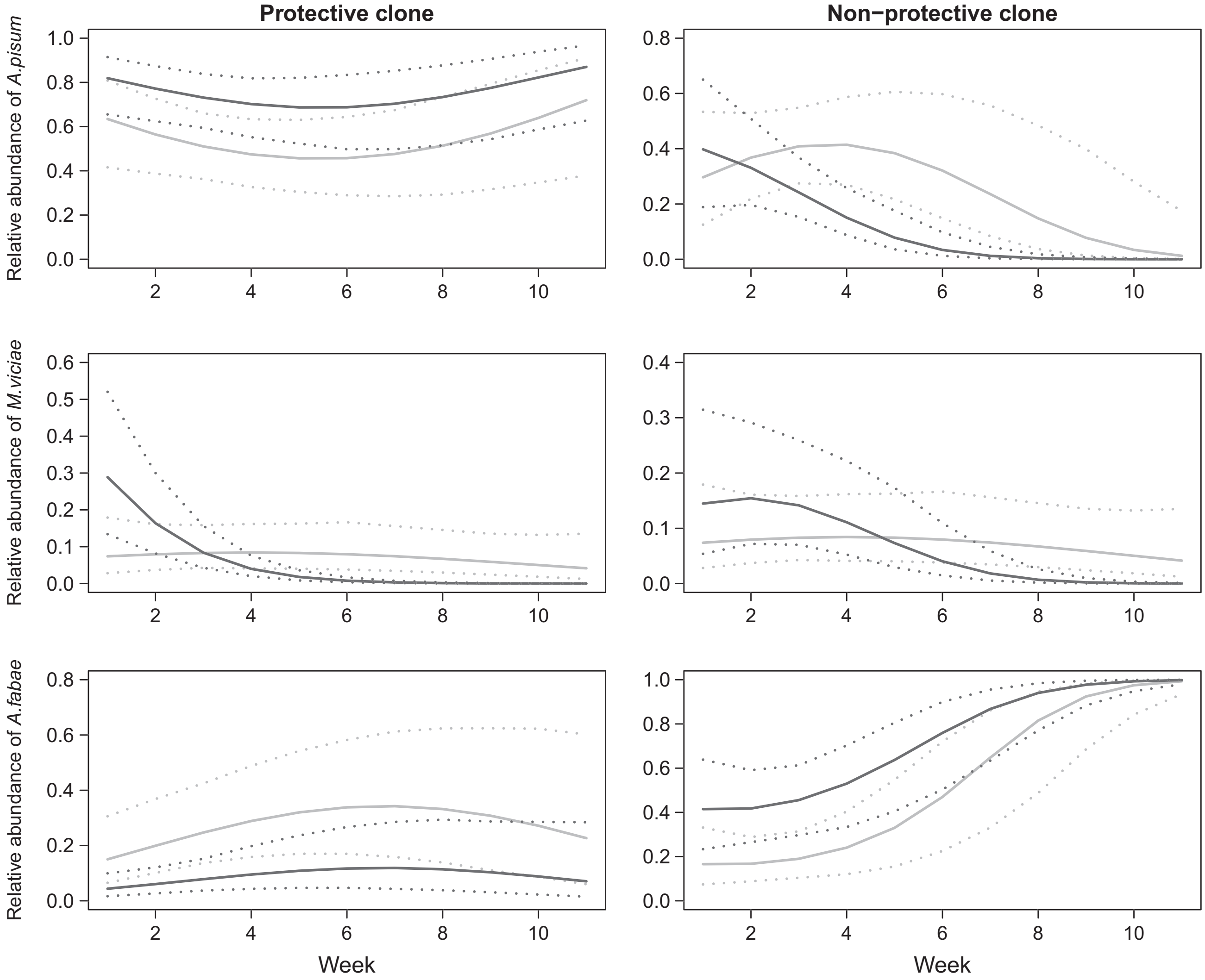

Figure 4 Relative aphid abundance (solid line) and 95% confidence intervals (dotted line) for model predictions in communities where the aphid clone (collected on *Medicago* and protected, or collected on *Ononis* and non-protected) and the symbiont *Hamiltonella defensa* (present or absent) were manipulated in *Acyrthosiphon pisum* aphids. Communities without the symbiont are represented with light grey lines, and those with the symbiont are represented with dark grey lines.

and parasitoid dynamics in all four treatments simultaneously to test the interaction term. Aphid clone had a significant effect on *A. pisum* cumulative numbers ($F_{1,27} = 58.06$, $P < 0.001$), and the symbiont effect varied between the two aphid clones used (symbiont × clone interaction: $F_{1,27} = 10.89$, $P < 0.001$; Table S3). Mean cumulative numbers of the parasitoid *A. ervi* were significantly affected by the presence of the symbiont ($F_{1,27} = 84.85$, $P < 0.001$), an effect that varied between aphid clones (symbiont × clone interaction: $F_{1,27} = 79.07$, $P < 0.001$; Table S3). In a mixed model with all fixed factors (week, week squared, symbiont presence and aphid clone), the percent variance explained by the symbiont and aphid clone was, respectively, 0.84 and 47.69% in *A. pisum* models, and 40.24 and 4.17% in *A. ervi* models.

The effect of *A. pisum* clonal differences at the community level

Given the observed difference in *A. pisum* clonal performance, we predicted that the other two aphid species and their parasitoids would be at an advantage in communities with the slower growing clone. There were no differences in the cumulative numbers of *M. viciae* or *A. fabae* aphids, or their associated parasitoids *A. megourae* and *L. fabarum*, in communities with the two *A. pisum* clones (*M. viciae:* $F_{1,9} = 2.30$, $P = 0.164$; *A. fabae:* $F_{1,9} = 1.03$, $P = 0.336$; *A. megourae*: $F_{1,9} = 2.60$, $P = 0.141$; *L. fabarum:* $F_{1,9} = 0.14$, $P = 0.722$; Table S1; Figs. 2 and 3). At the beginning of the experiment, the relative density of *M. viciae* was significantly larger in treatments with the slower growing *A. pisum* clone, although densities were similar towards the end of the experiment (clone × week interaction $Z = 2.48$, $P = 0.0133$; Table S2; Fig. S4). The relative density of *A. fabae* was not affected by *A. pisum* clone ($Z = -0.85$, $P = 0.393$; Table S2; Fig. S4) nor was the probability of aphid persistence (*M. viciae:* $\chi^2_2 = 3.55$, $P = 0.061$; *A. fabae:* $\chi^2_2 = 3.11$, $P = 0.078$; Table S1; Fig. 5: solid lines in panels A2, A3, A5 & A6). There was, however, a difference in the probability of parasitoid persistence; both *A. megourae* and *L. fabarum* where

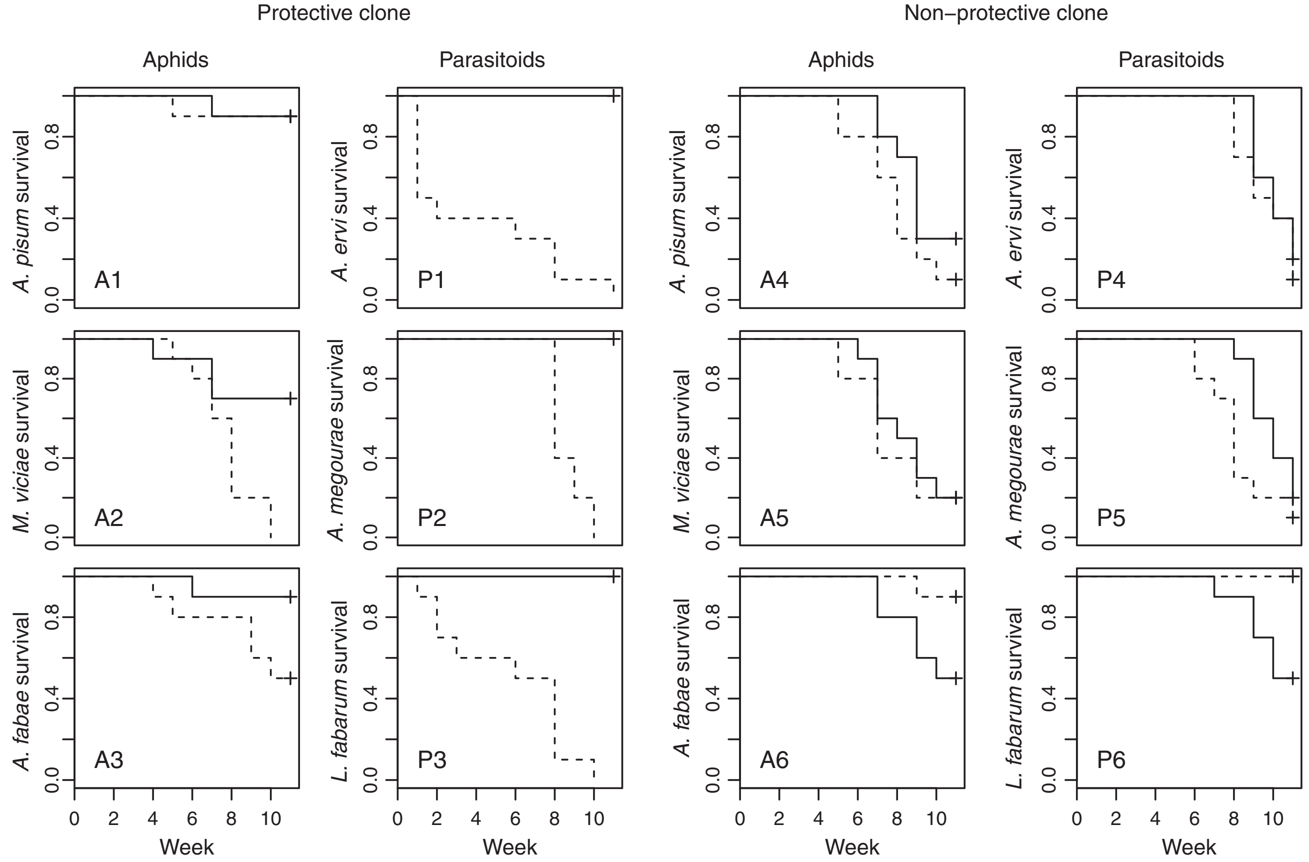

Figure 5 Persistence of the six species in communities where the aphid clone (collected on *Medicago* and protected, and collected on *Ononis* and non-protected) and the symbiont *Hamiltonella defensa* (present or absent) were manipulated in *Acyrthosiphon pisum* aphids. The *y*-axis represents the proportion of microcosm cages in which each species survived. Dashed lines represent communities with the symbiont present, and solid lines represent those without the symbiont.

significantly more likely to go extinct in cages with the slower growing aphid clone (*A. megourae:* $\chi^2_2 = 13.11$, $P < 0.001$; *L. fabarum:* $\chi^2_2 = 6.33$, $P = 0.012$; Table S1; Fig. 5: solid lines in panels P2, P3, P5 & P6). This was the opposite of what we expected. To test the interaction between the symbiont and *A. pisum* clone, aphid and parasitoid dynamics were analysed within a single factorial framework. Mean cumulative numbers of *M. viciae* aphids and their associated parasitoid *A. megourae* were significantly affected by the symbiont in *A. pisum* aphids (*M. viciae:* $F_{1,27} = 9.70$, $P = 0.004$; *A. megourae*: $F_{1,27} = 9.89$, $P = 0.004$), but not by *A. pisum* clone (*M. viciae:* $F_{1,27} = 0.11$, $P = 0.919$; *A. megourae*: $F_{1,27} = 0.54$, $P = 0.470$; Table S3). The symbiont effect was consistent between the two clones as revealed by the non-significance of the interaction terms, which were removed from the models. Mean cumulative numbers of the aphid *A. fabae* and its associated parasitoid *L. fabarum* were not affected by the symbiont in *A. pisum* aphids (*A. fabae:* $F_{1,27} = 0.18$, $P = 0.677$; *L. fabarum*: $F_{1,27} = 0.30$, $P = 0.587$; Table S3). Mean cumulative numbers of the aphid *A. fabae* were not affected by *A. pisum* clone ($F_{1,27} = 1.61$, $P = 0.215$), but cumulative numbers of parasitoid *L. fabarum* were ($F_{1,27} = 9.08$, $P = 0.006$; Table S3). For these two species, the symbiont effect varied between the two *A. pisum* clones (symbiont × clone interaction in *A. fabae:* $F_{1,27} = 15.54$, $P < 0.001$; *L. fabarum*: $F_{1,27} = 14.76$, $P < 0.001$; Table S3). In a mixed model with all fixed factors (week, week squared, symbiont presence and aphid clone), the percent variance explained by the symbiont and the aphid clone was, respectively, 4.28 and 3.61% for *M. viciae* models, 15.80 and 44.06% for *A. fabae* models, 4.40 and 2.11% for *A. megourae* models, and 4.01 and 92.24% for *L. fabarum* models.

Behavioural experiment

Contrary to our prediction, the number of *A. pisum* hosts attacked by *A. ervi* was not affected by the presence of non-host aphids ($\chi^2_2 = 3.43$, $P = 0.329$, Fig. S5). However, *A. ervi* attacked non-hosts, particularly *M. viciae* aphids. We analysed *A. ervi* attacks excluding control plants that harboured exclusively *A. pisum* aphids, and found that in the presence of the non-host *A. megourae*, the parasitoid attacked this host more often than its own host (treatment effect: $\chi^2_2 = 4.34$, $P = 0.114$; non-hosts effect: $\chi^2_2 = 0.51$, $P = 0.473$; interaction term: $\chi^2_2 = 10.65$, $P = 0.005$; Fig. S5).

DISCUSSION

Our study shows that manipulation of an endosymbiotic bacterium in an aphid species can affect the long-term dynamics of an experimental community consisting of three aphid species feeding on the same resource and their associated specialist parasitoid wasps. We manipulated the presence of the endosymbiotic bacterium *H. defensa*, in a pea aphid, *A. pisum*, clone where it confers resistance against the parasitoid *A. ervi*. This defensive phenotype led to higher *A. pisum* densities and the exclusion of its specialist parasitoid which became extinct in all experimental communities. Manipulating the bacterial strain that provided no protection against *A. ervi* did not significantly affect the density of *A. pisum* aphids and their associated parasitoids. As demonstrated previously for both aphids and *Drosophila*, the protective effect of a defensive symbiont commonly leads to higher host abundances in the presence of natural enemies (Oliver *et al.* 2008; Jaenike & Brekke 2011), and we show here that for a particular combination of aphid genotypes and symbiont strains, this protection also occurs in a more complex community with potentially strong interspecific competition at the herbivore trophic level.

The presence of the defensive symbiont in *A. pisum* also affected the dynamics and persistence of other species in the community. The larger numbers of *A. pisum* aphids when the symbiont was present did not lead to a reduction in the absolute density of the other two aphids, *M. viciae* and *A. fabae*, but it did reduce their relative abundance. More dramatically, their two parasitoids *A. megourae* and *L. fabarum* became extinct in all replicates when the protective symbiont was present, but survived in all replicates when it was absent. An explanation for this is suggested by previous work which has shown that the abundance of related non-host species can affect the efficiency of parasitoids searching for their specific host species (reviewed by van Veen & Godfray 2012). For example, by combining experimental microcosm experiments (involving the aphids *A. pisum*, *M. viciae* and the parasitoid *A. ervi*) with population modelling, van Veen *et al.* (2005) demonstrated that in the presence of non-host *M. viciae* the parasitoid *A. ervi* had a lower per-capita attack rate (a form of density-dependent interference). Increasing the density and diversity of non-host aphids in the environment has been shown to markedly reduce foraging efficiency in the parasitoids *A. megourae* and *L. fabarum* (Kehoe *et al.*, 2016). In the wasp behaviour experiment, we have also found that non-hosts aphids alter parasitoid foraging. In the presence of the non-host aphid *M. viciae*, the parasitoid *A. ervi* attacks this species more often than its own host *A. pisum*. In this experiment, wasps were tested for 10 minutes and in a simple scenario, and it is likely that in a more complex community and over the parasitoid's lifespan, these interactions will reduce foraging efficiency. We therefore hypothesise that in the current experiment, the decreased relative frequency of their hosts led to parasitoid wasps spending more time examining, rejecting and attacking unsuitable hosts, so that their searching efficiency and hence reproductive rate declined to a level at which the population could not sustain itself and extinction ensued. An important question for further research is to find out whether such behaviours only occur in confined conditions such as laboratory cages or whether they are relevant in the field.

The density of *A. pisum* thus affects the interaction between *A. megourae* and *M. viciae*, and *L. fabarum* and *A. fabae*, which are indirect effects since no direct resource–consumer (trophic) relationships are involved. The different parasitoid species can be considered to be connected by positive indirect interactions and the loss of one leads to an extinction cascade. A related example of the consequences of the loss of positive indirect interactions has recently been demonstrated in similar experimental aphid communities. Sanders *et al.* (2013) found that removal of one parasitoid species released its host from top-down control, and triggered the extinction of other indirectly linked parasitoid species. Compared to that study, we found extinction cascades were triggered earlier and in a larger proportion of replicates. A potential explanation for this is that Sanders *et al.* (2013) manipulated the aphid–parasitoid interaction by removing parasitised aphids (mummies) and this reduced parasitoid populations. In our study, however, the protective effect of the symbiont reduced the population growth of *A. ervi* parasitoids, but at the same time prevented the death of the attacked aphids.

We studied the effect of the symbiont on aphid–parasitoid communities in two different *A. pisum* clones: one naturally infected with a *H. defensa* strain that confers on its host a high level of parasitoid protection and the other with no known effect on parasitic wasps (Mclean & Godfray 2015). Contrary to our expectations, the presence of a non-protective symbiont affected the density of *A. fabae* aphids and its associated parasitoid *L. fabarum*, and in some cases aphid and parasitoid extinctions occurred, though the differences were not significant. Interpreting these results is complicated by differences in the intrinsic growth rates of the two aphid clones in the absence of symbiont. Although not an initial goal of our experiment, this led us to predict that extinction cascades would be triggered in communities with the faster growing clone. In fact, we found the opposite, suggesting that extinction cascades can be triggered not only when *A. pisum* comes to dominate the community but also when this species gets outcompeted by *M. viciae* and *A. fabarum*. Although our study was limited to two different *A. pisum* clones, these results also suggest that not all *A. pisum* genotypes facilitate the long-term stability of the community, and future work is therefore needed to unveil which particular traits promote stabilising positive indirect interactions. These traits might be influenced by the genotype of the herbivore or its symbiont composition, and might affect the insect susceptibility to natural enemies or traits related to plant exploitation. Herbivory can result in species-specific changes in plant morphology and physiology that through plant-mediated indirect effects have cascading consequences for other organisms in the community (Stam *et al.* 2013). Long-term community experiments can help us understand how indirect interactions involving higher or lower trophic levels modulate interactions among herbivorous species and ultimately affect the stability of terrestrial communities. At the evolutionary level, although aphid colonies are often composed of a single clonal lineage (Vantaux *et al.* 2011), aphids have also been used to show that natural enemy pressure rapidly selects for specific genotypes (Turcotte *et al.* 2011). It would be very interesting to explore evolutionary processes in more complex communities such as the one described here.

Understanding the factors that promote stability and diversity in natural communities is a topic of great relevance at a time when human activities threaten many natural ecosystems

(Barnosky *et al.* 2012; Cardinale *et al.* 2012). Our work reinforces the idea that direct and indirect interactions involving consumers and their prey or hosts are important in maintaining diversity in insect communities, and reveals that facultative insect symbionts can modulate the strength of these interactions in important ways. So far, little attention has been paid to the role of insect symbionts in this context, although we believe their consequences can be far-reaching. There are several examples of facultative symbionts in herbivorous insects that enable their hosts to spread geographically, either through the effects they have on their host's food-plant utilisation or their susceptibility to natural enemies (reviewed by Frago *et al.* 2012). For example, a genotype of the whitefly *Bemisia tabaci* is spreading around the globe partly due to a mutualism with a virus, which suppresses host-plant resistance (Li *et al.* 2014). Bark and ambrosia beetles can also become more serious pests by acquiring novel fungal associates that allow them to switch from attacking dead to live trees (Hulcr & Dunn 2011). In *Drosophila neotestacea*, the acquisition of a defensive endosymbiont in the genus *Spiroplasma* provided protection from a parasitic nematode and allowed certain matrilines to spread across central Canada (Cockburn *et al.* 2013).

With the proviso that our experiments took place in population cages and not in the field, and that a single protective strain of *H. defensa* was tested, our study shows that microbial symbionts can influence direct and indirect interactions between species and can thus trigger extinction cascades. Further work is needed in more natural situations to explore this phenomenon, especially to investigate the costs of symbiont carriage in the field and the complexities that may occur in communities containing many more hosts, natural enemies and symbionts than we have studied here. Thanks to the revolution in molecular biological techniques, the last two decades have seen a huge growth in our knowledge of the diversity of insect-associated microorganisms (Hansen & Moran 2014), but we are only beginning to explore the effects they may have at the community level. A deeper understanding of these effects will provide new insights into the structure and function of one of the most diverse types of community in terrestrial ecosystems, and into the forces that maintain diversity and the ecosystem services diversity provides (Hooper *et al.* 2012).

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AUTHOR CONTRIBUTIONS

DS and EF designed the study; DS and RK performed the research; AM created and characterised pea aphid lines; DS and EF analysed data; DS, FJFvV, HCJG and EF wrote the manuscript and all authors contributed to discussions and revisions.

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Defensive insect symbiont leads to cascading extinctions and community collapse

Dirk Sanders, Rachel Kehoe, Frank Van Veen, Ailsa McLean, H. Charles J. Godfray, Marcel Dicke, Rieta Gols, Enric Frago

Table and figure legends (Supporting information)

Table S1. Mixed effects model analyses, and survival analyses on aphid and parasitoids in communities where the aphid clone (collected on *Medicago* and protected, or collected on *Ononis* and non-protected) and the symbiont *Hamiltonella defensa* (present or absent) were manipulated in *Acyrthosiphon pisum* aphids. Analyses test symbiont effects in both *A. pisum* clones independently, and clone effects in replicates without symbionts. * d.f. are 1, 197 in *M. viciae* models, ** d.f. are 1, 198 in *L. fabarum* models.

Table S2. Mixed effects model analyses on aphid relative abundances in communities where the aphid clone (collected on *Medicago* and protected, or collected on *Ononis* and non-protected) and the symbiont *Hamiltonella defensa* (present or absent) were manipulated in *Acyrthosiphon pisum* aphids.

Table S3. Mixed effects model analyses on aphid and parasitoids in communities where the aphid clone (collected on *Medicago* and protected, or collected on *Ononis* and non-protected) and the symbiont *Hamiltonella defensa* (present or absent) were manipulated in *Acyrthosiphon pisum* aphids. Analyses test all four treatments simultaneously (i.e. symbiont and clone effects, and their interaction). * d.f. are 1, 28 in *M. viciae* model in clone or symbiont parameters, ** d.f. are 1,397 for *A. ervi* in week parameters.

Figure S4. Relative aphid abundance (solid line) and 95% confidence intervals (dotted line) for model predictions in replicates without symbionts where the aphid clone (collected on *Medicago* and fast-growing, or collected on *Ononis* and slow-growing) was manipulated in *Acyrthosiphon pisum* aphids. Communities with the slow-growing clone are represented with light grey lines, and those with the fast-growing clone are represented with dark grey lines.

Figure S5. Parasitoid attacks on host and non-host aphids. Mean number (±SE) of *Aphidius ervi* attacks on *Acyrthosiphon pisum* host aphids (dark grey bars), and on *Megoura viciae* and *Aphis fabae* non-host aphids (light grey bars). Treatments represent *A. pisum* aphids alone, or with one or both of the non-host aphids.

Table S1

	Parameters		*Acyrthosiphon pisum*		*Megoura viciae*		*Aphis fabae*	
Protective clone	Aphids	d.f. *	F-value	p	F-value	p	F-value	p
	Intercept	1, 198	**318.08**	**<.0001**	**103.59**	**<.0001**	**97.45**	**<.0001**
	Symbiont	1, 9	**6.93**	**0.027**	4.79	0.057	2.90	0.123
	Week	1, 198	**19.00**	**<.0001**	**108.45**	**<.0001**	0.66	0.418
	Week 2	1, 198	**17.09**	**<.0001**	**13.34**	**<.0001**	2.73	0.100
	Symbiont x Week	1, 197			**11.11**	**<.0001**		
	Parasitoids	d.f. **	*Aphidius ervi*		*Aphidius megourae*		*Lysiphlebus fabarum*	
	Intercept	1, 197	**125.72**	**<.0001**	**125.91**	**<.0001**	**23.29**	**<.0001**
	Symbiont	1, 9	**98.35**	**<.0001**	**5.22**	**0.048**	2.66	0.137
	Week	1, 197	**6.97**	**0.009**	**138.67**	**<.0001**	0.17	0.681
	Week 2	1, 197	**8.83**	**0.003**	2.14	0.145	0.12	0.732
	Symbiont x Week	1, 197	**5.89**	**0.016**	**8.07**	**0.005**		
Non-protective clone	Parameters		*Acyrthosiphon pisum*		*Megoura viciae*		*Aphis fabae*	
	Aphids	d.f.	F-value	p	F-value	p	F-value	p
	Intercept	1, 198	**197.66**	**<.0001**	**96.10**	**<.0001**	**96.65**	**<.0001**
	Symbiont	1, 9	3.51	0.094	1.68	0.227	**6.62**	**0.030**
	Week	1, 198	**84.90**	**<.0001**	**141.69**	**<.0001**	**4.53**	**0.035**
	Week 2	1, 198	1.45	0.229	**4.25**	**0.041**	0.48	0.488
	Parasitoids	d.f.	*Aphidius ervi*		*Aphidius megourae*		*Lysiphlebus fabarum*	
	Intercept	1, 198	**154.19**	**<.0001**	**117.02**	**<.0001**	**70.88**	**<.0001**
	Symbiont	1, 9	0.20	0.669	2.93	0.121	**5.44**	**0.045**
	Week	1, 198	**142.01**	**<.0001**	**213.83**	**<.0001**	0.47	0.493
	Week 2	1, 198	2.79	0.097	1.51	0.221	1.11	0.293
Clonal lineage (cured lines)	Parameters		*Acyrtosiphon pisum*		*Megoura viciae*		*Aphis fabae*	
	Aphids	d.f. *	F-value	p	F-value	p	F-value	p
	Intercept	1, 198	**217.26**	**<.0001**	**100.22**	**<.0001**	**106.05**	**<.0001**
	Clone	1, 9	**8.09**	**0.019**	2.30	0.164	1.03	0.336
	Week	1, 198	**34.22**	**<.0001**	**109.83**	**<.0001**	3.34	0.069
	Week 2	1, 198	3.91	0.050	**6.44**	**0.012**	0.43	0.515
	Symbiont x Week	1, 197			**30.20**	**<.0001**		
	Parasitoids	d.f. **	*Aphidius ervi*		*Aphidius megourae*		*Lysiphlebus fabarum*	
	Intercept	1, 197	**217.11**	**<.0001**	**134.46**	**<.0001**	**58.99**	**<.0001**
	Clone	1, 9	**6.23**	**0.034**	2.60	0.141	0.14	0.722
	Week	1, 197	**39.78**	**<.0001**	**140.21**	**<.0001**	0.79	0.377
	Week 2	1, 197	**7.43**	**0.007**	**3.98**	**0.047**	1.81	0.181
	Symbiont x Week	1, 197	**5.03**	**0.026**	**18.07**	**<.0001**		
Survival	Survival		*Acyrthosiphon pisum*		*Megoura viciae*		*Aphis fabae*	
	Parameters	d.f.	X^2	p	X^2	p	X^2	p
	Symbiont (protective clone)	1	0.00	0.970	**7.09**	**0.008**	3.45	0.063
	Symbiont (non-protective)	1	2.37	0.123	0.16	0.688	3.76	0.053
	Clonal lineage (cured lines)	1	**6.33**	**0.012**	3.55	0.061	3.11	0.078
			Aphidius ervi		*Aphidius megourae*		*Lysiphlebus fabarum*	
		d.f.	X^2	p	X^2	p	X^2	p
	Symbiont (protective clone)	1	**22.00**	**<.0001**	**21.40**	**<.0001**	**20.30**	**<.0001**
	Symbiont (non-protective)	1	0.49	0.486	3.10	0.078	**6.34**	**0.019**
	Clonal lineage (cured lines)	1	**13.22**	**<.0001**	**13.11**	**<.0001**	**6.33**	**0.012**

Table S2

	Parameters	*Acyrthosiphon pisum*				*Megoura viciae*				*Aphis fabae*			
		Estimate	Std	z-value	p	Estimate	Std	z-value	p	Estimate	Std	z-value	p
Protective clone	Intercept	0.91	0.61	1.51	0.132	**-2.64**	**0.69**	**-3.84**	**0.000**	**-2.14**	**0.61**	**-3.48**	**<0.0001**
	Symbiont (present)	0.96	0.51	1.89	0.058	**2.43**	**0.71**	**3.44**	**0.001**	**-1.35**	**0.60**	**-2.24**	**0.025**
	Week	**-0.40**	**0.20**	**-1.99**	**0.047**	0.12	0.23	0.53	0.597	**0.44**	**0.20**	**2.23**	**0.026**
	Week 2	**0.04**	**0.02**	**2.41**	**0.016**	-0.02	0.02	-0.83	0.406	**-0.03**	**0.01**	**-2.18**	**0.029**
	Symbiont x Week					**-0.81**	**0.12**	**-6.99**	**<0.0001**				

	Parameters	*Acyrthosiphon pisum*				*Megoura viciae*				*Aphis fabae*			
		Estimate	Std	z-value	p	Estimate	Std	z-value	p	Estimate	Std	z-value	p
Non-protective clone	Intercept	-1.34	0.77	-1.74	0.082	-0.89	0.68	-1.32	0.187	**-1.48**	**0.67**	**-2.21**	**0.027**
	Symbiont (present)	1.06	0.88	1.21	0.227	**-1.13**	**0.58**	**-1.97**	**0.049**	**1.27**	**0.53**	**2.38**	**0.017**
	Week	0.55	0.28	1.92	0.055	0.34	0.24	1.41	0.158	-0.20	0.27	-0.77	0.442
	Week 2	**-0.07**	**0.02**	**-3.37**	**0.001**	**-0.09**	**0.02**	**-4.28**	**<0.0001**	**0.07**	**0.02**	**3.29**	**0.001**
	Symbiont x Week	**-0.61**	**0.23**	**-2.64**	**0.008**								

	Parameters	*Acyrthosiphon pisum*				*Megoura viciae*				*Aphis fabae*			
		Estimate	Std	z-value	p	Estimate	Std	z-value	p	Estimate	Std	z-value	p
Clonal lineage	Intercept	0.48	0.43	1.128	0.259	**-2.24**	**0.67**	**-3.34**	**0.001**	**-1.73**	**0.45**	**-3.83**	**<0.0001**
	Clone (slow-growing)	**-0.74**	**0.31**	**-2.378**	**0.017**	**2.55**	**0.71**	**3.57**	**<0.0001**	-0.32	0.38	-0.85	0.393
	Week	-0.07	0.16	-0.441	0.659	-0.03	0.20	-0.16	0.870	0.20	0.17	1.17	0.242
	Week 2	-0.01	0.01	-0.564	0.573	0.00	0.01	-0.13	0.894	0.00	0.01	0.19	0.853
	Symbiont x Week					**-0.36**	**0.14**	**-2.48**	**0.013**				

Table S3

Paramenters		*Acyrtosiphon pisum*		*Megoura viciae*		*Aphis fabae*	
Aphids	d.f. *	F-value	p	F-value	p	F-value	p
Intercept	1, 398	**568.93**	**<.0001**	**336.83**	**<.0001**	**709.43**	**<.0001**
Symbiont	1, 27	1.61	0.216	**9.70**	**0.004**	0.18	0.677
Clone	1, 27	**58.06**	**<.0001**	0.11	0.919	1.61	0.215
Week	1, 398	**72.06**	**<.0001**	**224.57**	**<.0001**	**17.24**	**<.0001**
Week 2	1, 398	**13.57**	**0.000**	0.77	0.381	1.53	0.217
Symbiont x Clone	1, 27	**10.89**	**0.002**			**15.54**	**0.001**

		Aphidius ervi		*Aphidius megourae*		*Lysiphlebus fabarum*	
Parasitoids	d.f. **	F-value	p	F-value	p	F-value	p
Intercept	1, 398	**578.61**	**<.0001**	**288.41**	**<.0001**	**172.60**	**<.0001**
Symbiont	1, 27	**84.85**	**<.0001**	**9.89**	**0.004**	0.30	0.587
Clone	1, 27	**14.72**	**<.0001**	0.54	0.470	**9.08**	**0.006**
Week	1, 398	**89.77**	**<.0001**	**317.46**	**<.0001**	0.27	0.605
Week 2	1, 398	**3.08**	**<.0001**	**0.03**	**0.868**	1.96	0.163
Symbiont x Clone	1, 27	**79.07**	**<.0001**			**14.76**	**0.001**

Figure S4

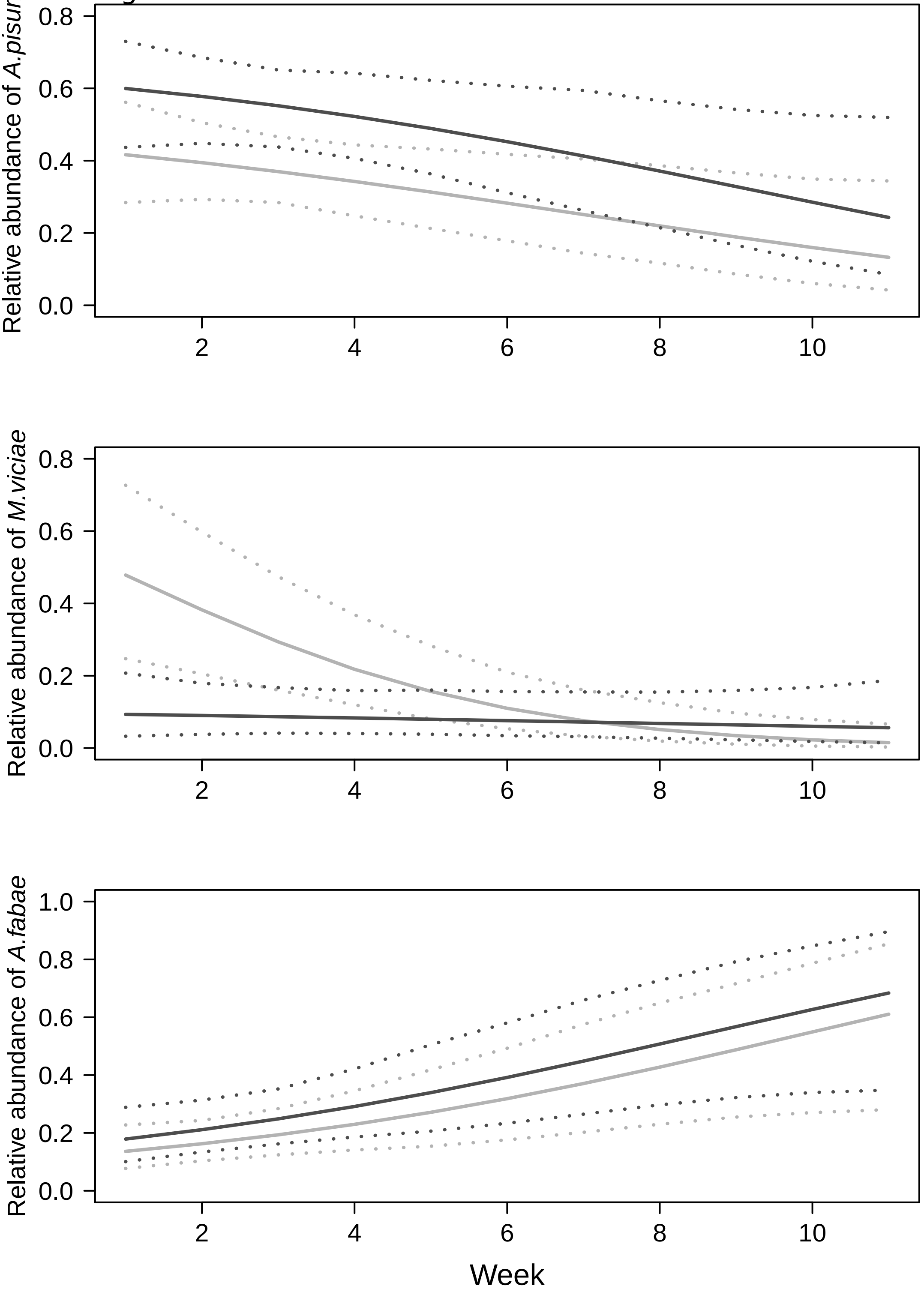
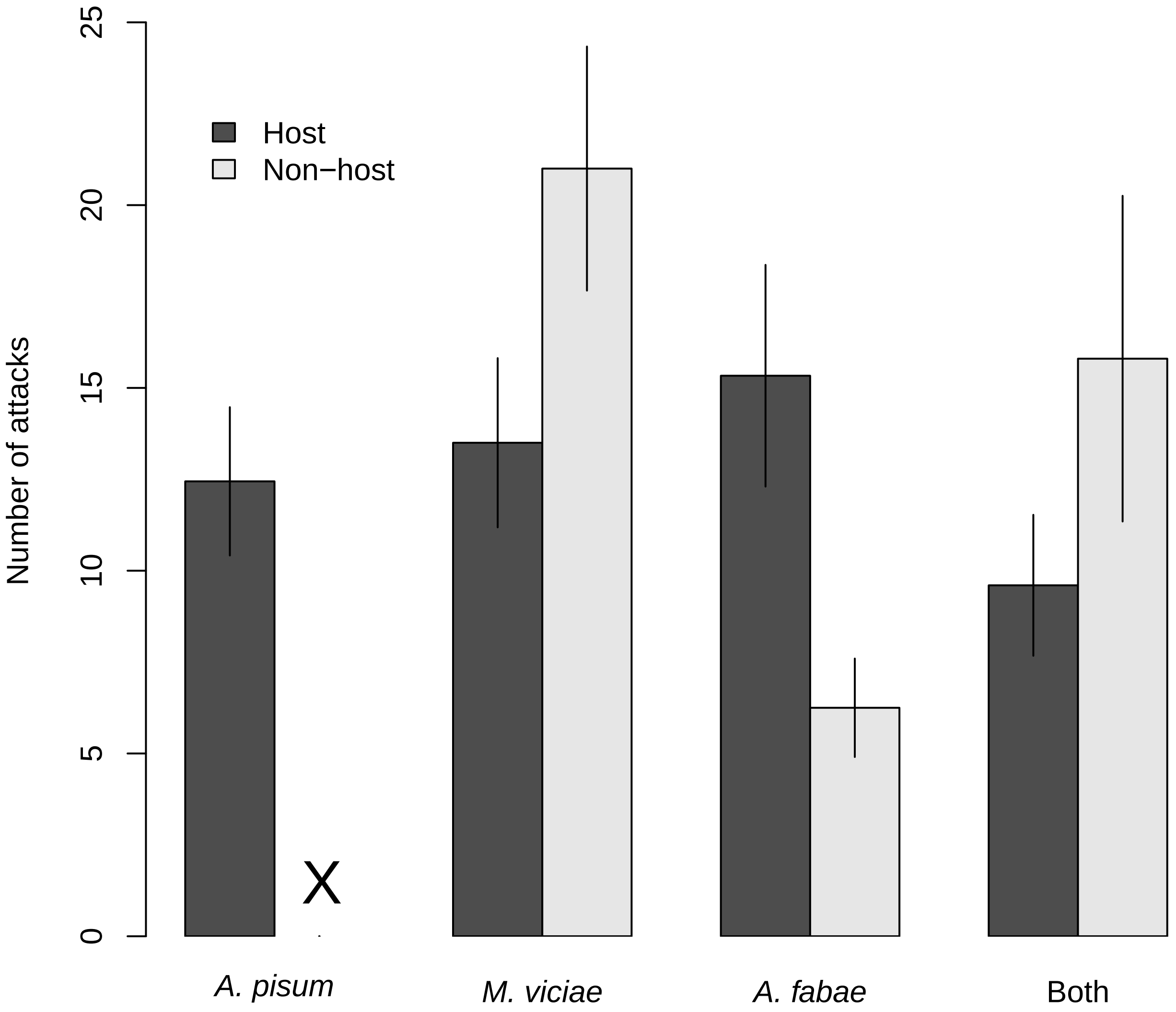

Relative abundance of *A.pisum*
0.0
0.2
0.4
0.6
0.8
2
4
6
8
10
Relative abundance of *M.viciae*
0.0
0.2
0.4
0.6
0.8
2
4
6
8
10
Relative abundance of *A.fabae*
0.0
0.2
0.4
0.6
0.8
1.0
2
4
6
8
10
Week

Figure S5


Number of attacks
0
5
10
15
20
25
Host
Non−host
X
A. pisum
M. viciae
A. fabae
Both

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Current Opinion in
Insect Science

Interactions between parasitoids and higher order natural enemies: intraguild predation and hyperparasitoids

Enric Frago[1,2]

Parasitoids kill and live at the expense of their hosts, but they also serve as food for intraguild predators and hyperparasitoids. Natural enemy diversity can thus challenge herbivore suppression by parasitoids, but this depends on the ecological niches of the species involved and their functional diversity. The spatial context is another important layer of complexity, particularly in areas with reduced habitat complexity and increased fragmentation. Parasitoids have evolved strategies to locate their host, but this can be affected by risk of intraguild predation or hyperparasitism. To better understand these interactions we need more long-term experiments and trophic-web studies. This will provide fundamental knowledge, improve pest control, and allow ecologists to better predict the impact of human activities on species extinctions.

Addresses
[1] CIRAD, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France
[2] Laboratory of Entomology, Wageningen University, Droevendaalsesteeg 1, Building 107, PO Box 16, 6700AA Wageningen, The Netherlands

Corresponding author: Frago, Enric (enric.frago@gmail.com)

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Introduction

Parasitoids kill and live at the expense of their hosts, but they can also serve as food for other organisms like intraguild predators or hyperparasitoids. Intraguild predation occurs when competing natural enemies prey on each other, and when they occur between parasitoids and predators, the parasitoid is always the subordinate species (i.e. the intraguild prey) as their larvae can be eaten by the intraguild predator [1,2]. Hyperparasitism involves parasitoids that attack other parasitoid species [3]. In plant-based ecosystems, these interactions have been extensively studied because they have major consequences for the control of pest species, primary productivity and nutrient cycling [4–6]. Intraguild predation and hyperparasitism are ubiquitous in natural trophic webs [3,7–10], which contrasts with theoretical work that suggests restricted scenarios for species coexistence [6,11,12]. During the past few decades, both theoretical and experimental research has tried to explain this discrepancy. Parasitoids of herbivorous insects have played a relevant role in these developments because many population dynamics models have been inspired by host–parasitoid systems, and because parasitoids have been used to test their predictions (e.g. [13]).

There is an intense debate about whether herbivore suppression is enhanced at larger natural enemy diversity. The main reason is that although herbivore suppression often correlates positively with natural enemy diversity, natural enemies may engage in antagonistic interactions like intraguild predation or hyperparasitism [4]. In this review, I show that although natural enemy diversity might challenge herbivore suppression by parasitoids, this depends on the ecological niches of the species involved. I also discuss the importance of long-term, multi-generational experiments, and of trophic web studies in this context. The spatial context is another layer of complexity that affects intraguild predation and hyperparasitism, especially due to human impacts that reduce habitat complexity and increase fragmentation. I also hypothesise that in complex communities, host location has driven selection on parasitoid behaviour, but these strategies also include avoiding antagonistic interactions. Finally I discuss future avenues for research and their applied implications in view of the global changes imposed by human activities (Figure 1).

Diversity impacts on herbivore suppression

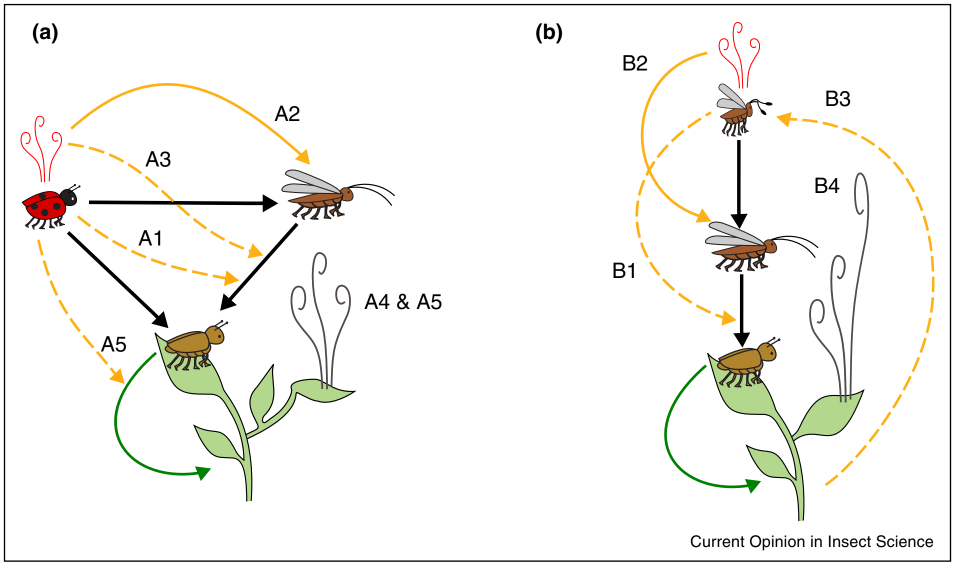
Many studies exploring intraguild predation in parasitoids have been restricted to a single insect generation, and have studied simple webs composed of three species [1,7,8]. These studies have been useful to understand the behavioural strategies underlying species interactions, but they have limited ability to predict long-term community dynamics. For example, over multiple generations, parasitoids and intraguild predators can exploit their hosts at different moments of the host life cycle, or parasitoids can develop when intraguild predators are less active [13]. Studies with complex communities in natural ecosystems have also revealed emergent impacts of diverse predatory guilds on herbivore suppression, which are difficult to predict from pairwise interactions [14••,15,16]. These studies, and work with other animal taxa, have revealed that niche complementarity is an important characteristic to consider [17,18]. Functionally diverse predatory guilds might partition the prey resource leading to increased herbivore suppression [19,20]. Given their different life histories, complementarity is thought to be particularly strong between parasitoids and intraguild predators [19]. For instance, in an experiment with the aphid *Eriosoma lanigerum*, consistent aphid suppression was only achieved when the parasitoid *Aphelinus mali* was paired with a diverse guild of generalist predators [14••]. Even within parasitoid guilds, niche complementarity may depend on parasitoid host range [20], facultative hyperparasitism [21,22], and potentially on phylogenetic diversity [23••].

Hyperparasitoids have the potential to release herbivorous insects from their primary parasitoids [3,9]. Theoretical models and field data, however, suggest that in the long-term hyperparasitoids can sometimes promote herbivore population suppression by stabilising insect–parasitoid dynamics [3,11]. As with intraguild predation, exploring complex scenarios and long-term dynamics is needed to understand hyperparasitoid ecology. In a long-term field study, *Cotesia vestalis* was the main parasitoid of the diamondblack moth, *Plutella xylostella*. When hyperparasitism levels were high, however, other primary parasitoids were the dominant ones [24]. Quantitative trophic webs have also provided detailed information on how hyperparasitoid networks regulate primary parasitoid populations (e.g. [10,25]). Parasitoids of bigger aphid species, for instance, might represent stronger links in trophic webs because they host a larger number of hyperparasitoids, with female biased sex-ratios [26,27]. At the evolutionary level, another aphid study has revealed that trophic webs are phylogenetically constrained, from the plant to the hyperparasitoid level [28]. These examples reveal that although trophic web studies usually consider species identities, interactions can be importantly modulated by species traits and their evolutionary history.

The role of spatial complexity

Intraguild prey usually suffer less predation in structured habitats, potentially due to reduced encounter rates with

Figure 1



Current Opinion in Insect Science

Parasitoids often engage in antagonistic interactions with higher order natural enemies like **(a)** intraguild predators and **(b)** hyperparasitoids. Direct trophic effects involve a consumer-resource interaction (black solid lines), whereas direct trait-mediated effects involve changes in the behaviour or morphology of the interacting species (yellow solid lines). Interactions among species can be indirect if they are mediated by at least a third species (yellow dashed lines). Herbivory has a direct effect on plant traits or defensive state (solid green lines), and also an indirect effect on parasitoid foraging through herbivore-induced plant volatiles (grey vapour lines). (a) Intraguild predators (represented by a ladybird) can reduce herbivore suppression by parasitoids by preying on parasitoid larvae (A1). Herbivore suppression, however, is influenced by the functional niche of the intraguild predator, and by the diversity of the community of natural enemies, at both the species and the phylogenetic level. Parasitoids detect and avoid chemical cues from intraguild predators (A2), and these responses can have consequences for host–parasitoid dynamics (A3). Risk of intraguild predation can alter parasitoid attraction to herbivore-induced plant volatiles (A4). Risk of predation can also affect the way herbivores feed on plants and hence plant volatile induction, with consequences for parasitoid foraging (A5). (b) Hyperparasitoids (top trophic level) attack primary parasitoids and can affect herbivore-parasitoid dynamics (B1). This effect, however, depends on the trophic web of herbivores, primary parasitoids and hyperparasitoids, and on the traits and evolutionary history of the species involved. Primary parasitoids detect and avoid chemical cues from hyperparasitoids (B2). Herbivory can affect plant traits or defensive state, and these changes can cascade up to the hyperparasitoid level (B3). Hyperparasitoids can use herbivore-induced plant volatiles to locate their hosts (B4). For both intraguild predation and hyperparasitism, these interactions are influenced by spatial complexity, at both the plant and the landscape level.

the predator, and increased availability of refuges (e.g. [29,30]). For instance, exploitation of hosts on different plant substrates might explain coexistence of the parasitoids *Aphytis melinus* and *Encarsia perniciosi*, which attack the California red scale *Aonidiella aurantii*, but at the same time feed on each other's larvae [30]. Some experiments also suggest that one way parasitoids avoid intraguild predators is by exploiting patches where hosts seek refuge from the predator [31•]. Aphid parasitoids, for example, avoid plants colonised by ladybird predators [32], or they can benefit by dispersing to patches with lower aphid densities if ladybirds aggregate in response to aphid numbers [33]. Human-modified landscapes have been often used to explore spatial effects on natural enemy diversity and herbivore suppression [34], but only few studies have considered how such diversity might also increase intraguild predation. In cabbage fields, although intercropping increased parasitism on diamondback moth larvae, *Plutella xylostella*, it did not affect intraguild predation by the invasive fire ant *Solenopsis invicta* [35]. Another study also exposed cabbage herbivores to natural enemies in a gradient of habitat complexity. The authors found that when birds were excluded, moth control by insect predators and parasitoids increased with habitat complexity. Intraguild interactions, however, were larger in complex habitats because this effect was impaired when birds were not excluded [36].

Although little explored, spatial complexity can allow the coexistence of different hyperparasitoid species, as suggested in a study with the hyperparasitoids of *Cotesia glomerata*. This parasitoid was attacked by four hyperparasitoid species but at different rates in the plant canopy, or close to the ground [37]. As found with other insect groups, landscape complexity usually correlates positively with parasitoid diversity and abundance (e.g. [38]), but these effects are probably stronger in hyperparasitoids as they are located at the top of many terrestrial food webs. For this same reason, hyperparasitoids are an ideal group to test ecological theory. Food-web theory, for instance, predicts that higher trophic levels are more susceptible to disturbances [39], and this has been demonstrated in a landscape modification gradient studying aphids, and their associated parasitoids and hyperparasitoids [40]. Island theory predicts that species richness correlates with area, and that the slope of this correlation steepens at higher trophic levels. This prediction too has been recently supported by estimating the diversity of lepidopterans and their associated parasitoids and hyperparasitoids, in twenty islands that varied in size [41••].

Trait-mediated indirect effects: parasitoids avoiding risk

In ecological communities, many interactions do not involve resource–consumer (trophic) relationships, but changes in the behaviour or morphology of the interacting species. These interactions are known as trait-mediated, and are often indirect if one species affects a second through at least a third one [10]. The past decades have revealed the intricate ways parasitoids use information to locate their hosts, and as intraguild prey, parasitoids use chemical information to avoid patches with high risk of predation. Host–parasitoid interactions can therefore be modulated by the presence of the intraguild predator, a type of trait-mediated indirect effect. Several studies have revealed that predator cues are avoided by parasitoids (e.g. [42]). These cues can reduce parasitoid efficiency by increasing the time adult wasps need to handle hosts [43], or the number of eggs they lay per individual host [44]. Parasitoids too can affect the interaction between an intraguild predator and its prey, for example when parasitoids trigger defensive behaviours in their hosts that expose them to predators [45]. In complex communities, these effects can have far-reaching consequences. In a study with a community composed of two aphid species that share a parasitoid, chemical cues from the ladybird *Coccinella septempunctata* reduced the time the parasitoid *Aphidius ervi* spent foraging on the plant. This released aphid populations from top-down control ultimately affecting the long-term dynamics of the community [42].

It has long been suggested that one way herbivores can benefit from hyperparasitoids is through trait-mediated indirect effects if hyperparasitoids trigger density-dependent avoidance behaviours in primary parasitoids [3,7,9]. Several studies have explored the chemical ecology of these interactions and have identified the chemical cues that primary parasitoids use to avoid successful attack by hyperparasitoids (reviewed in [31•]). Hyperparasitoid cues can also trigger increased reproduction in aphids, as they may signal low risk of primary parasitism [46]. By combining experimental and theoretical methods, a recent work has provided novel insights into how hyperparasitoids can shape oviposition behaviour and meta-population dynamics in the parasitoid *Hyposoter horticola*, which attacks the gregarious butterfly *Melitaea cinxia*. To avoid density-dependent hyperparasitism, when adult wasps locate a larval cluster, only a portion of hosts are attacked [47••]. Risk of hyperparasitism and predation has therefore shaped parasitoid foraging behaviour, but we are only beginning to understand the intricate strategies parasitoids use to avoid their own natural enemies. For instance, evidence is mounting that parasitoids manipulate the behaviour of their hosts to use them as bodyguards (reviewed in [48]). Parasitoids can also mimic other organisms to avoid predation, as in the parasitoid *Gelis agilis*, which has evolved chemical, morphological and behavioural traits to mimic the sympatric ant *Lasius niger* [49].

Trait-mediated indirect effects: induced plant defences

Parasitoid hosts are often inconspicuous, and to locate them adult wasps can use indirect cues from plants.

Parasitoids home in on volatile compounds that plants emit after insect attack (i.e. herbivore-induced plant volatiles). These plant responses are known as indirect plant defences, as opposed to direct defences, which aim at poisoning or repelling the herbivore. Plant attraction of parasitoids through indirect plant defences has been intensively studied, and evidence suggests that they can be impaired by risk of intraguild predation. The aphid parasitoid *Lysiphlebus fabarum* was attracted by the volatile blends of bean plants infested with *Aphis fabae* aphids, but attraction was reduced when plants also bore ladybird predators [32]. A more recent study with the root-feeding fly *Delia radicum*, found that the parasitoid *Trybliographa rapae* was attracted to volatiles from cabbage plants infested with the fly, but attraction was reduced when the roots were also inoculated with an entomopathogenic fungi [50••]. Neither study, however, identified whether wasp behaviour was affected by changes in plant volatiles, or by volatiles that were directly emitted by the intraguild predator, a question that would be very interesting to explore. We know, for example, that changes in prey behaviour in response to predation risk have important consequences for the functioning of insect-plant communities (e.g. [51]). Hence, it is possible that predation risk affects the way herbivores feed on plants, and ultimately plant defences which attract parasitoids [52].

Several studies have demonstrated that plant nutritional quality can have a cascading effect on hyperparasitoids. This has been demonstrated in experiments with plant varieties that differ in their chemical properties [27,53], or by inducing changes through root herbivory [54] or herbivore egg deposition [55•]. Hyperparasitoids too use plant volatiles to detect their hosts. When feeding on cabbage plants, larvae of the butterfly *Pieris rapae* trigger the emission of volatiles that attract the butterfly's natural enemies. When the caterpillars are parasitised by *Cotesia glomerata*, however, volatile blends are affected. Both in the field and in the laboratory the hyperparasitoid *Lysibia nana* is able to discriminate these blends, which allows this to locate and attack its hosts [56,57••]. These studies reveal the intricate ways plant volatiles can meditate community interactions.

Future perspectives and applied implications

Most research on intraguild predation and hyperparasitism aims at enhancing pest control. As antagonistic interactions, intraguild predation and hyperparasitism are expected to reduce pest suppression, but few, if any, long-term experimental studies have been able to demonstrate this. The focus on pest control implies that most research has been done in agricultural ecosystems, and an important amount of work has also been done in laboratory or greenhouse settings. This limits several studies to simplified ecosystems, and to hymenopteran parasitoids of herbivorous insects. More research is therefore needed in natural ecosystems, with other insect guilds like detritivores, and with other parasitoid groups like tachinid flies. Particularly in diverse tropical regions, field experiences might require intense taxonomic work. This can be facilitated by the use of molecular techniques, which allow inferring predator diets from their guts, and parasitoid trophic-webs from host insects [58].

Human activities have unprecedented impacts on species extinctions, and on the services biodiversity provides to our society. To predict these impacts, one of the main challenges faced by ecologists is to understand the structure and diversity of networks of interacting species. These networks should consider indirect interactions, as up to 80% of the species extinctions, for example, are triggered by the loss of indirect links [59]. Parasitoids play a very relevant role in mediating such effects [10], but indirect effects mediated by plants [52] and insect-associated microbes [60] also need to be considered. Global changes, together with habitat loss and fragmentation, are a primary threat to wildlife. These changes have consequences for species persistence and dispersal traits. Field experiments where dispersal is not restricted are thus needed to unveil these impacts on higher-order parasitoid interactions. For example, changes in trophic-webs can be assessed over large geographical areas, and during multiple host generations. These experiments might be labour intensive, and experimental exclusion of specific insect guilds (as in [36]) might be a shorter-term solution. This knowledge will have important applied implications, but will also provide exciting insights into the ecological mechanisms, and evolutionary processes that shape terrestrial communities.

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- • of special interest
- •• of outstanding interest

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