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Plant–insect interactions under bacterial influence: ecological implications and underlying mechanisms

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Abstract

Plants and insects have been co-existing for more than 400 million years, leading to intimate and complex relationships. Throughout their own evolutionary history, plants and insects have also established intricate and very diverse relationships with microbial associates. Studies in recent years have revealed plant- or insect-associated microbes to be instrumental in plant–insect interactions, with important implications for plant defences and plant utilization by insects. Microbial communities associated with plants are rich in diversity, and their structure greatly differs between below- and above-ground levels. Microbial communities associated with insect herbivores generally present a lower diversity and can reside in different body parts of their hosts including bacteriocytes, haemolymph, gut, and salivary glands. Acquisition of microbial communities by vertical or horizontal transmission and possible genetic exchanges through lateral transfer could strongly impact on the host insect or plant fitness by conferring adaptations to new habitats. Recent developments in sequencing technologies and molecular tools have dramatically enhanced opportunities to characterize the microbial diversity associated with plants and insects and have unveiled some of the mechanisms by which symbionts modulate plant–insect interactions. Here, we focus on the diversity and ecological consequences of bacterial communities associated with plants and herbivorous insects. We also highlight the known mechanisms by which these microbes interfere with plant–insect interactions. Revealing such mechanisms in model systems under controlled environments but also in more natural ecological settings will help us to understand the evolution of complex multitrophic interactions in which plants, herbivorous insects, and micro-organisms are inserted.

Key words: Herbivores, host–microbe interactions, microbial induced responses, plant defences, symbionts, trophic networks.

Introduction

Insects and plants have been co-existing for more than 400 million years and have been engaged since then in a running arms race: while insects have to face plant defences and evolve strategies to overcome them, plants tend to reduce herbivory through diverse mechanisms.

In parallel, plants and insects have established different types of relationships with microbial associates that could influence the outcomes of the interactions. Microbes may modulate plant primary and secondary metabolisms and/or plant defence systems against insects for the benefit of either plants or insects. Microbes may also change insect biology, including metabolism and behaviour, and have a significant influence on plant–insect interactions. Microbes can act directly or indirectly on the traits of insects that participate in habitat and resource exploitation and/or in survival under stressed conditions. Progress has been made in describing the

microbial diversity associated with plants and insects, but the specific roles of these microbial communities for plants or insect herbivores remain mostly uncertain. Nevertheless, recent developments in sequencing methods and molecular tools are advancing our understanding of the influence of microbes on plant–insect interactions at the molecular level. This article places plants in the centre of multitrophic interactions and reviews our current knowledge on the plant–insect interactions that are influenced by microbial associates of either plants or insects. First, we present the diversity, structure, and location in the host of bacterial communities associated with plants and insects. Next, we review the dynamics of host–microbe associations and their ecological and evolutionary implications in the context of plant–insect interactions. Finally, we examine the known mechanisms by which microbes interfere with plant–insect interactions. Although many microbes such as fungi and viruses can also influence plant–insect interactions, we will focus here on the impact of plant- or insect-associated bacteria on plant–insect interactions.

Microbial diversity associated with plants and herbivorous insects

Characterization of microbial diversity in plant and insect hosts

Besides the well-studied symbioses in plants (e.g. mycorrhizal and nitrogen-fixing bacterial mutualists) and insects (e.g. *Buchnera aphidicola*, the aphid obligatory symbiont), recent researches that employ advanced technologies have highlighted a plethora of previously hidden microbial associates. In insects, this concerns the growing field of research on facultative symbionts (also referred to as secondary or accessory symbionts as opposed to obligatory or primary symbionts) and, to a lesser extent, the inhabitants of the digestive tract, referred to as gut associates. These microbial communities, although not essential for the host's reproduction and survival, may considerably affect many aspects of their host's ecology, behaviour, and physiology, such as traits associated with plant utilization, protection against natural enemies, or responses to climate changes (Oliver *et al.*, 2010). Eventually, it is whole microbial communities associated sustainably or temporarily with plants and insects that are now scrutinized. Plant and insect microbiomes represent the microbial communities that interact superficially or internally with their hosts. Assessing the diversity and structure of microbial communities associated with plants and insects has benefited from the emergence and rapid spread of next-generation sequencing (NGS) technologies, allowing the detection of the taxonomic diversity of microbes in many ecosystems and environmental conditions. NGS technologies realize high-throughput sequencing of PCR-amplified taxonomic genes (e.g. 16S rRNA gene for bacteria, 18S rRNA gene for fungi), whole (meta)-genome sequencing, and whole (meta)-transcriptomics. Coupled with adapted bioinformatic tools and specific databases, these genomic datasets reveal the presence and abundance of microbial taxa associated with their hosts in a given environment, including those taxa of low prevalence. Furthermore, the transcription datasets of microbes will allow indirect assessment of their biological functions.

Bacterial communities associated with herbivorous insects

The literature on insect-associated bacterial diversity is growing rapidly, but we still require information from some important groups of herbivorous insects (e.g. Lepidoptera). The general picture emerging from this body of work is that herbivorous insects harbour microbial communities of limited diversity, dominated by a few taxa (Colman *et al.*, 2012; Jones *et al.*, 2013). While mammalian guts are generally colonized by about 1000 taxa, *Drosophila* species harbour no more than 30 sequence-based differentiated taxa or operational taxonomic units (OTUs) on average (Chandler *et al.*, 2011). Two recent papers analysing the microbial communities of insect species from different orders also concluded that bacterial species diversity is low (10–15 OTUs on average per insect) and supported the hypotheses that both evolutionary history and feeding habits structure insect microbial communities (Colman *et al.*, 2012; Jones *et al.*, 2013). For example, xylophagous insects harbour the richest gut bacterial communities with about 103 OTUs per sample, while leaf-feeders are intermediate with about 38 OTUs per sample. Sap-feeding insects such as aphids, psyllids, and whiteflies have the poorest microbial diversity, with no more than three to seven OTUs per sample (Colman *et al.*, 2012; Russell *et al.*, 2013; Jing *et al.*, 2014). For these insects, most individuals are dominated by primary and secondary symbionts that are specifically associated with these different groups of sap feeders. It has been hypothesized that low diversity in microbial communities of phloemophagous insects could be due to several non-mutually exclusive explanations including: (i) exclusive nutrition on a generally microbe-free medium, the phloem sap: (ii) the immunity system of the host aided by its heritable symbionts; and (iii) antagonism between resident symbionts and transient bacteria (Jing *et al.*, 2014). Hemipteran insects, which encompass a range of phloem, xylem, and mesophyll feeders but also a few species feeding on whole leaf tissues, offer a good system for testing which factors shape their associated microbial communities and, more specifically, whether host diet could be considered as a key driving force of microbial diversity.

Proteobacteria, and in particular Enterobacteriaceae, are the most prevalent microbial associates of herbivore insects. This major group of bacteria includes a wide range of obligatory and facultative insect symbionts such as *Buchnera*, the endosymbiont of aphids, *Carsonella*, the endosymbiont of psyllids, *Portiera*, the endosymbiont of whiteflies, and SOPE, the *Sitophilus oryzae* (rice weevil) principal endosymbiont (Moran *et al.*, 2008). *Acetobacter* and *Lactobacillus* are also commonly found in *Drosophila*, in addition to Enterobacter (Chandler *et al.*, 2011). Firmicutes and Bacteroidetes are strongly associated with xylophagous insects, especially those feeding on decayed wood (Colman *et al.*, 2012). Mollicutes, and *Spiroplasma* in particular, are also very common inhabitants of insects.

Bacterial communities reside in different parts of the host. Obligatory symbionts are hosted in insect-derived structures called bacteriocytes. Facultative symbionts can occupy different locations within the host such as in secondary bacteriocytes, sheath cells in the periphery of primary bacteriocytes, and haemolymph. Many bacterial associates are also harboured in different niches of the insect gut and can play important nutritional roles either directly or indirectly (Dillon and Dillon, 2004). Other bacteria such as *Wolbachia* and *Spiroplasma* infect insect reproductive organs and alter the host sex ratio in many ways (Engelstadter and Hurst, 2009). However, the same bacterial taxa could also increase their host's fitness and thus function as both mutualists and reproductive manipulators (Teixeira *et al.*, 2008; Himler *et al.*, 2011). There is also increasing evidence that bacterial communities can colonize the salivary glands of their insect's hosts, with important consequences for plant–insect interactions including diseases vectored by herbivorous insects but also plant metabolic reconfiguration for insect nutritional purposes (Kaiser *et al.*, 2010; Body *et al.*, 2013). For example, *Candidatus* Liberibacter asiaticus, the causal agent of Huanglongbing or citrus greening, the most destructive citrus disease worldwide, is found in salivary glands of psyllid vectors (Ammar *et al.*, 2011). *Candidatus* Liberibacter asiaticus is also found in salivary glands of mealybugs, another group of phloem sap feeders, but this strain is different from the one transmitted by psyllids and does not cause disease in host plants (Pitino *et al.*, 2014). Flavescence dorée, a severe grapevine disease, is caused by *Candidatus* Phytoplasma vitis, a member of the Mollicutes. This bacterium is spread by the leafhopper, *Scaphoideus titanus* (Cicadellidae) and is found in salivary glands of its vectors (Marzorati *et al.*, 2006). The leaf-miner caterpillar *Phyllonorycter blancardella*, working through an endosymbiotic bacteria (*Wolbachia*), alters the phytohormonal profiles of the leaf to create an enhanced nutritional microenvironment (Kaiser *et al.*, 2010; Body *et al.*, 2013). In this system, accumulation of bacteria in salivary glands can be observed (D. Giron, W. Kaiser, J. Bertaux, M. Body, S. Guyot and D. Bouchon, unpublished data).

Bacterial communities associated with plants

In contrast to insects, plants are hosts of an impressive diversity of microbes including pathogens, commensals, and beneficial or potentially beneficial associates. Mycorrhizal fungi and nitrogen-fixing bacteria are well-known examples of mutualist microbes, but other endophytic fungi and bacteria provide important benefits to their host plants through a wide range of biological functions (Bulgarelli *et al.*, 2013). Typically, bacterial communities associated with plants fall into two categories, depending on their location: bacteria that colonize below-ground tissues (or the rhizosphere) and bacteria that colonize above-ground tissues (or the phyllosphere). Microbial communities of the phyllosphere can be as abundant as 10^6–10^7 bacterial cells cm^{-2} of leaf area, whether living on or within leaves (Humphrey *et al.*, 2014). The root endosphere and the rhizosphere (in contact with the soil) host an even richer bacterial ecosystem, with 10^4–10^8 bacterial cells g^{-1} of root tissues and 10^6–10^9 bacterial cells g^{-1} of rhizospheric tissues (Bulgarelli *et al.*, 2013). In a recent study on the *Arabidopsis thaliana* microbiome, 600–1000 OTUs were detected with 454 pyrosequencing of the 16S rRNA gene (Bodenhausen *et al.*, 2013), with this number depending on the plant tissues. However, 21 OTUs were particularly abundant, representing 67% of the overall sequences. Besides leaf and root tissues, little is known about microbial communities in other plant organs (Bulgarelli *et al.*, 2013; Turner *et al.*, 2013), in particular in plant vessels, which constitute the primary interface with sap-feeding insects.

Among the most common bacterial taxa associated with plants are Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria (Bodenhausen *et al.*, 2013; Bulgarelli *et al.*, 2013). While Bacteroidetes are equally abundant in roots and leaves, Proteobacteria dominate the phyllosphere. More generally, bacterial communities of above and below ground differ in both their diversity and structure. It has been hypothesized that this difference in microbial communities results from distinct selective processes between the phyllosphere and rhizosphere. The composition of bacterial communities of the rhizosphere seems to be determined primarily by the combined influence of root exudates, which drive the differentiation of the soil biome in the rhizosphere, and by host genotype-dependent factors. By contrast, phyllosphere communities appear to be selected at the immediate leaf surface only (Bulgarelli *et al.*, 2013). This hypothesis, however, awaits further validation, as little is known about the source of bacterial communities in the phyllosphere and about the role of other biotic (such as insect herbivory) and abiotic factors that could also shape community structure below and above ground (Badri *et al.*, 2013; Humphrey *et al.*, 2014).

Ecological and evolutionary patterns of host–microbe interactions

The complex dynamics of host–microbe interactions

Insects and plants are associated with a large spectrum of microbes that are engaged in long-term and intimate relationships with their hosts, or have more temporary or occasional interactions with them. These microbes interact with their hosts in many ways from parasitic to mutualistic relationships, but the nature of these interactions may change according to ecological factors, and the effect of microbial communities is very often context dependent. For example, the Enterobacter *Erwinia* sp., which infects the thrips *Frankiniella occidentalis*, can be beneficial or detrimental for its host depending on which plant the thrip is feeding on (de Vries *et al.*, 2004). Also, insects and plants are infected by a wide range of parasites, but under certain circumstances, these parasitic interactions may evolve towards beneficial relationships. This shift from parasitism to mutualism generally arises over evolutionary timescales but can also be very fast: in just 20 years, *Drosophila simulans* populations infected by *Wolbachia* have gained a 10% fecundity increase over uninfected females, possibly due to a change in the endosymbiont genome (Weeks

et al., 2007). *Rickettsia*, another insect symbiont, which normally manipulates insect host reproduction, has been found to spread rapidly (6 years) in natural populations of the sweet potato whitefly, *Bemisia tabaci*, in the southwestern USA, and to considerably increase the performance of infected whiteflies compared with uninfected ones (Himler *et al.*, 2011). Furthermore, plant pathogens transmitted by insects may sometimes have beneficial effects on insect vectors, whether direct (e.g. increased performances) or indirect (e.g. increased plant attraction) (Beanland *et al.*, 2000; Kluth *et al.*, 2002; Sugio *et al.*, 2011). On the other hand, a plant pathogen may reduce its virulence to achieve a long-term association with the host plants (Pagán *et al.*, 2014). Thus, there is no evolutionary gap between parasitic and mutualistic symbionts and between insect symbionts and insect-transmitted plant pathogens (Frago *et al.*, 2012).

Additionally, microbes are engaged not only in two-way interactions with their host plants or host insects but also in three-way interactions between plants, microbes, and insects, with evolutionary and co-evolutionary consequences (Biere and Bennett, 2013; Biere and Tack, 2013). It has been shown, for example, that aphid–barley interactions are dependent on the genotypes of the interacting species as well as of the rhizosphere bacteria (Tetard-Jones *et al.*, 2007, 2012). More generally, microbial communities are inserted in complex trophic networks where they could exert their influence and be influenced at different levels (Fig. 1) (Pieterse and Dicke, 2007).

Microbes as drivers of diversification and plant specialization in herbivorous insects

Microbes can bring key innovations to their hosts, allowing the exploitation of new environments (also referred to as ecological opportunities). The acquisition of symbionts in diverse groups of herbivorous insects has promoted host-plant specialization and adaptive divergence (Janson *et al.*, 2008). For example, the establishment of symbiotic associations with different bacterial taxa has allowed the diversification of sap-feeding insects and the colonization of virtually all plants by specialized or generalist species. Each hemipteran group is host for a specific bacterial symbiont such as *Buchnera* for aphids, *Portiera* for whiteflies, *Carsonella* for psyllids, and *Tremblaya* for coccids (Hansen and Moran, 2014). Sometimes, two symbionts are required for nutritional provisioning as for the conifer aphid, *Cinara cedri*, which harbours a strain of *B. aphidicola* with a highly reduced genome, and *Serratia symbiotica*, which is apparently shifting from a status of facultative to obligate endosymbiont (Lamelas *et al.*, 2011). This is also the case for some leafhoppers (i.e. *Homalodisca vitripennis* in association with both *Sulcia muelleri* and *Baumannia*
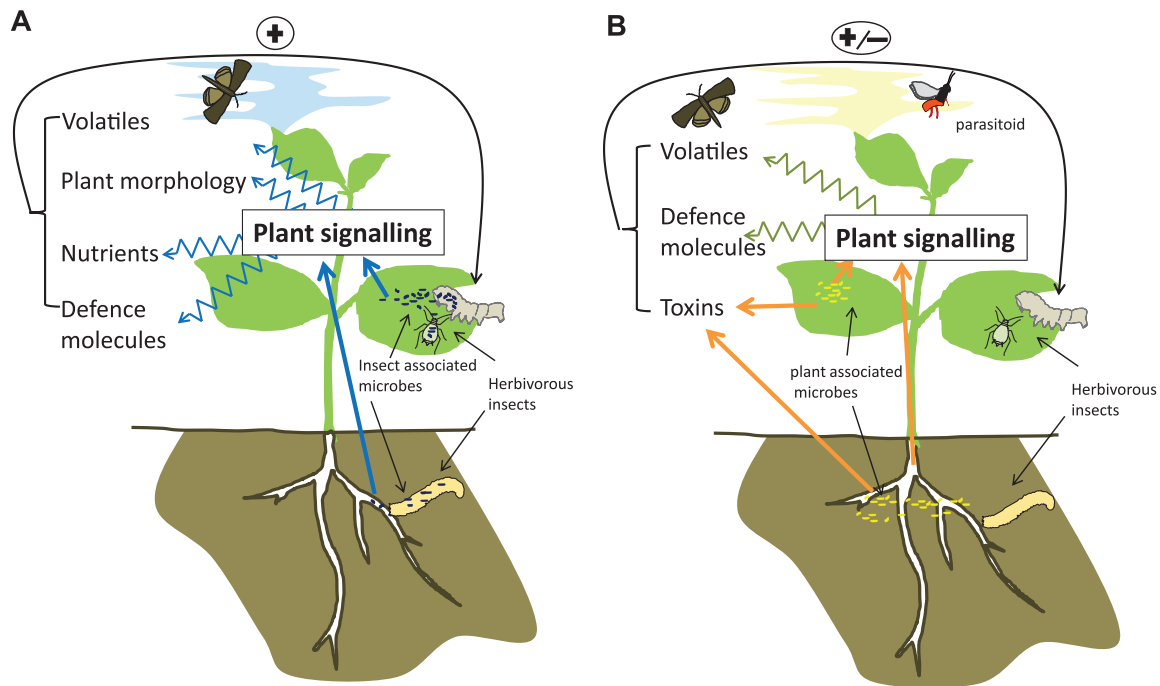

Fig. 1. Schematic representation of bacteria-mediated plant–insect interactions. (A) Bacterial symbionts associated with herbivorous insect (shown as blue rod shapes) can be localized in various body compartments including salivary glands, gut, bacteriocytes, or haemolymph. Some bacteria-derived proteins and bacteria themselves are secreted into plants. Some bacteria can also systemically infect plants and recognized as plant pathogens. The insect-associated bacteria interfere with various plant signalling processes resulting in alterations of volatile composition, plant morphology, nutrients, and defence-related compound production. These alterations often have positive effects on insect fitness and attraction of adult insects. However, see Chaudhary *et al.* (2014) as an example of a negative impact of insect-associated bacteria on the host insect fitness. (B) Plant-associated bacteria (shown as yellow rod shapes), localized either in the rhizosphere or in the phyllosphere, interfere with plant signalling pathways leading to alterations of plant volatile composition and defence-related molecule production. These alterations can have either a positive or a negative impact on insect fitness. Note that the effect of the bacteria-mediated plant alteration may depend on the feeding guild (leaf chewing or sap feeding) and specialization (specialist or generalist) of the insect. Some plant-associated bacteria can also produce toxins that act directly against insect herbivores.

cicadellinicola) and mealybugs (i.e. *Planococcus citri* in association with both *Tremblaya princeps* and *Moranella endobia*). Microbial partners and communities may also contribute to plant specialization of their insect hosts. For example, the chestnut weevil, *Curculio sikkimensis*, shows different symbiotic communities depending on its host plants (Toju and Fukatsu, 2011). For some herbivorous insects, performances on host plants are directly determined by the genotype of their symbionts: the exchange of the symbiont *Candidatus* Ishikawaella capsulata between two stinkbug species of the genus *Megacopta* modifies their pest status on soybean (Hosokawa *et al.*, 2007). The pea aphid, *A. pisum*, is often associated with facultative symbionts in addition to the obligate symbiont *B. aphidicola.* The distribution of these secondary symbionts in aphid populations shows strong links with the host aphid biotypes, each of which feeds on specific host plants (Simon *et al.*, 2003; Ferrari *et al.*, 2012; Henry *et al.*, 2013). Thus, it is hypothesized that these facultative symbionts help the adaptation of aphids to specific host plants. Indeed, Tsuchida *et al.* (2004) revealed that *Regiella insecticola*, one of the several facultative symbionts of *A. pisum*, increases the aphid's fecundity on white clover (*Trifolium repens*) but not on vetch (*Vicia sativa*). However, other studies have reported neutral or negative effects of *R. insecticola* on aphid fecundity on clover (Ferrari *et al.*, 2007; Leonardo, 2004; McLean *et al.*, 2011). As these studies used different genotypes of aphid, *R. insecticola*, and different clover species, the positive effect of *R. insecticola* on aphid seems to be context dependent. Interestingly, the same *R. insecticola* strain that increased the pea aphid fecundity on clover increased the survival and reproduction of the non-host aphid *Megoura crassicauda* in clover (Tsuchida *et al.*, 2011). Thus, this specific *R. insecticola* strain can increase the performance of two distinct aphid species on white clover by an unknown mechanism. Although *Hamiltonella defensa* of the whitefly, *B. tabaci*, is reported to increase the host's fitness on low-nitrogen diets (Su *et al.*, 2013), *H. defensa* and other facultative symbionts of *A. pisum* do not dramatically increase aphid fitness on specific host plants (Leonardo, 2004; Ferrari *et al.*, 2007; McLean *et al.*, 2011). Hence, the *R. insecticola* story seems to be the exception rather than the rule. The facultative symbionts of *A. pisum* may increase aphid performance in the host-plant-specific environment or may have compatibility issues with specific plant–aphid combinations.

Transmission of microbial communities associated with insects and plants

Transmission patterns of plant or insect-associated microbes are important drivers of plant–microbe, insect–microbe, and plant–microbe–insect interactions. Plant symbionts are generally acquired by their hosts through regular infection from the environment (e.g. nitrogen-fixing rhizobia invade regularly new legume plants from the soil) and are mostly facultative. However, obligatory symbiosis with *Burkholderia* sp. bacteria inhabiting specialized leaf nodules has been reported in several plant species of the genus *Psychotria*

(Rubiaceae). In this case, bacteria are transmitted vertically between plant generations and individuals devoid of symbionts fail to develop (Lemaire *et al.*, 2012). Obligate symbionts of insects are strictly maternally inherited, while their facultative symbionts, although transmitted vertically with a high fidelity, occasionally jump between close or distant host species (Oliver *et al.*, 2010; Hansen and Moran, 2014). In the plataspid stinkbug, *Megacopta punctatissima*, a frequent pest of soybean, obligate symbionts are deposited in capsules next to the eggs on the leaf surface, and newborn insects become infected by ingesting the capsule's content (Hosokawa *et al.*, 2005). *Riptortus clavatus*, another stinkbug species, acquires a beneficial gut symbiont of the genus *Burkholderia* from the soil at every generation, this insect–microbe relationship being comparable to symbioses between plants and soil-associated microbes, such as legume–*Rhizobium* and alder–*Frankia* mutualisms (Kikuchi *et al.*, 2007). The routes of horizontal transmission are not always clear. In insects, occasional paternal transmission of facultative symbionts may occur, as in the pea aphid, allowing symbiont spread among host populations and sometimes across closely related species through intra- and interspecific hybridization events (Moran and Dunbar, 2006). Parasitic wasps, which are common natural enemies of herbivorous insects, may also transfer symbionts from one host to another through their ovipositor, which acts as a dirty needle (Chiel *et al.*, 2009; Gehrer and Vorburger, 2012). Finally, a few studies have hypothesized that symbionts of herbivores may be acquired via the plant, although this possibility lacks robust experimental evidence. This hypothesis is well supported in the case of the whitefly *B. tabaci* for which it has been demonstrated that horizontal transmission of *Rickettsia*, one of its facultative symbionts, can be plant mediated (Caspi-Fluger *et al.*, 2012). This route of transmission is, however, not surprising, as it is frequently used by plant pathogens that are spread by insect vectors.

Gene transfers mediated by plant–insect–microbe interactions

One important evolutionary and functional consequence of plant–microbe–insect interactions is the possibility of lateral gene transfer (LGT) either between the host and its symbiont(s) or between microbial associates of plants or insects (Janson *et al.*, 2008; Hansen and Moran, 2014). This mechanism constitutes a source of variation for traits involved directly or indirectly in nutrition or stress response in plants and insects. For example, *Hypothenemus hampei*, an important pest of coffee crops, integrated a *Bacillus* mannanase gene in its genome, allowing the exploitation of coffee berries as a new ecological niche (Acuna *et al.*, 2012). Similarly, it has been shown that cellulase genes enabling plant cell degradation have a microbial origin in both termites and plant nematodes (Danchin *et al.*, 2010; Todaka *et al.*, 2010). In the mealybug *Planococcus citri*, LGT from diverse bacteria to the host genome enables a tripartite nested symbiosis involved in nutrient provisioning of the insect (Husnik *et al.*, 2013). Gene transfers between plants and/or insect microbial associates

are also possible. A putative amino acid transporter has been acquired by the plant pathogen *Candidatus* Liberibacter from the beta-proteobacterium *Candidatus* Profftella armatura, an endosymbiont of its psyllid vector (Nakabachi *et al.*, 2013). There is limited evidence for LGT in chloroplast and nuclear genomes of plants (Richardson and Palmer, 2007). For example, *Agrobacterium rhizogenes* has donated genes, some functional, to members of its host genus, *Nicotiana* (Suzuki *et al.*, 2002). Another putative case of bacterium-to-plant nuclear genome transfer involves the acquisition of glutathione biosynthesis genes from an alpha-proteobacterium (Copley and Dhillon, 2002). These LGT events will alter the traits of the host plants or insects and may change the level of associations between the microbes and their hosts.

Mechanisms of plant–insect interactions under bacterial influence

In this section, we first introduce basic knowledge on plant defences to herbivorous insects in order to facilitate an understanding of mechanisms of plant–insect interactions that are influenced by bacterial symbionts, which are then developed. Note that there are several excellent articles that explore current knowledge on the microbes that facilitate insect nutrient uptake from the host plants (Douglas, 2013; Hansen and Moran, 2014). Therefore, we focus here on the bacteria that interfere with plant–insect interactions by mechanisms that do not directly involve nutrient provisioning.

Plant hormones mediate major plant defence responses against herbivorous insects

Plants produce various chemicals and volatiles to repel attackers. Constitutive production of molecules that are toxic to insects can be costly or damaging to plants; thus, plants often protect themselves by inducing production of such molecules only when they need to. Upon insect attack, plants trigger a complex signalling process to induce a resistance reaction and to produce defensive molecules that deter or harm the attackers (Wu and Baldwin, 2010). Jasmonic acid (JA)- and ethylene (ET)-mediated responses are induced by necrotic pathogens and tissue-damaging insects and trigger defence reactions that are effective against these attackers including the production of protease inhibitors and secondary metabolites such as terpenes. Salicylic acid (SA) mediates a signalling pathway that is antagonistic to the JA/ET signalling pathways. SA-mediated defence signalling is induced by biotrophic pathogens and also by some sap-feeding insects (hemipterans) such as aphids and whiteflies, which require living plant cells to maintain an active feeding process (Moran and Thompson, 2001; Zarate *et al.*, 2007). Interestingly, however, the JA signalling pathway but not the SA signalling pathway mediates plant defence responses that are effective against some sap-feeding insects such as a whitefly and a leafhopper (Zarate *et al.*, 2007; Sugio *et al.*, 2011). In the case of plant defence against aphids, the situation is more complex. JA seems to mediate plant defence against aphids, as demonstrated in various experiments

(Thompson and Goggin, 2006; de Vos *et al.*, 2007). However, other defence pathways are also involved in plant defence against aphids (Louis and Shah, 2013). Overall, it is safe to say that the JA signalling pathway has a central role in defending plants from a variety of insect herbivores, although its importance varies depending on each interaction. Thus, manipulation of the hormone balances or the signalling processes leads to the manipulation of plant–insect interactions, and we will review such cases below.

Plant-associated bacteria can interfere with plant defence signalling

Plant roots are associated with a diverse microbial community of soil-borne bacteria (and fungi) that can be beneficial or pathogenic to the plants. Some growth-promoting rhizobacteria are known to induce so-called induced systemic resistance (ISR) against microbial pathogens and herbivorous insects and contribute to the promotion of plant growth (Van Wees *et al.*, 2008; Pineda *et al.*, 2010). ISR is mediated by priming of defence genes and often involves responsiveness of the plant to the phytohormones JA and ET (Van der Ent *et al.*, 2009*b*). ISR-primed plants induce faster and stronger expression of cellular defence responses upon pathogen or insect attack, resulting in an enhanced level of resistance to the aggressors (Pozo *et al.*, 2008; Van der Ent *et al.*, 2009*a*). Interestingly, the effectiveness of ISR depends on the degree of specialization of the herbivorous insects. *Pseudomonas fluorescens* WCS417r induced ISR in *Arabidopsis* and significantly reduced the weight gain of the generalist caterpillars *Spodoptera exigua* that fed on the above-ground tissues compared with the caterpillars that fed on the control plant, while there was no significant effect on the specialist caterpillar *Pieris rapae* (Van Oosten *et al.*, 2008). In line with this report, various soil microbial communities applied to *Arabidopsis* roots were shown to diminish the weight gain of the generalist caterpillar *Trichoplusia ni* (Badri *et al.*, 2013). In contrast, *P. fluorescens*-induced ISR in *Arabidopsis* increased the performance (weight gain and intrinsic rate of increase) of the generalist aphid *Myzus persicae* but had no effect on the specialist aphid *Brevicoryne brassicae* (Pineda *et al.*, 2013*b*). Furthermore, *P. fluorescens* colonization changed the composition of *Arabidopsis* volatiles induced by *Myzus persicae* infestation and made the plant less attractive to the aphid parasitoid *Diaeretiella rapae* (Pineda *et al.*, 2013*a*). Thus, *P. fluorescens* colonization in *Arabidopsis* roots has both positive and negative effects for the plant in terms of plant–insect interactions: it increases the resistance of the plant against generalist chewing insects (e.g. lepidopterans), but at the same time it increases the susceptibility to generalist sap-feeding insects (e.g. hemipterans) while there is no significant effect on specialized insects that belong to both feeding groups.

The microbial communities associated with above-ground plant tissues can also influence the interactions between plant and insect by altering plant defence signalling pathways. In case of *P. syringae*, which produces the JA mimic coronatine, the bacterial infection induces JA signalling-mediated

systemic resistance to the cabbage looper *Trichoplusia ni* (Cui *et al.*, 2005). However, in the absence of coronatine, the bacterium induces systemic-induced susceptibility to *Trichoplusia ni* by inducing microbe-associated molecular pattern-triggered SA signalling and suppressing the defences mediated by JA as a consequence (Cui *et al.*, 2005; Groen *et al.*, 2013). Consistent with these laboratory studies, a field survey of phyllosphere bacteria and the feeding damage caused by a chewing insect, *Scaptomyza nigrita*, in the bittercress (*Cardamine cordifolia*) showed positive correlation between *P. syringae* abundance and the herbivore damage. However, the abundance of other bacteria such as *Pedobacter* spp. and *P. fluorescens* was negatively correlated with herbivory, suggesting intricate interactions of plant defence responses operating against the bacteria and the herbivore (Humphrey *et al.*, 2014).

Plant-associated bacteria can produce toxins

Some plant-associated microbes may interfere directly with insect fitness by producing toxins. *Bacillus thuringiensis* is a Gram-positive, spore-forming bacterium that produces crystal proteins, Cry and Cyt, upon sporulation. The crystal proteins act as insecticides by forming pores in the epithelial midgut cells (Vachon *et al.*, 2012). Through the pores created, the bacteria gain access to the haemocoel, increase their population, and cause septicaemia, resulting in insect death. In addition to the crystal proteins, the bacteria employ additional toxins and various effectors that interfere with insect immunity and promote infection (Nielsen-LeRoux *et al.*, 2012). The bacterial spores can be found in the soil, the cadavers, and the phyllosphere, but the ecology of *B. thuringiensis* is not well understood. Recent studies have shown that the bacteria in the soil can colonize the growing plants and can be taken up by insects when they ingest the leaves of the plants (Bizzarri and Bishop, 2008; Monnerat *et al.*, 2009). Thus, *B. thuringiensis* seems to move between plants, insects, and soil in nature. The toxicities of the *B. thuringiensis*-colonized leaves depend most likely on the insects, bacterial strains, and host plants (Bizzarri and Bishop, 2008; Monnerat *et al.*, 2009).

Pseudomonas protegens (formerly known as *P. fluorescens*) strains CHA0 and Pf-5 are well-characterized rhizospheric bacteria that protect plants from microbial pathogens by secreting antimicrobial compounds (Haas and Keel, 2003; Loper and Gross, 2007). Recently, these bacteria were shown to encode the genomic locus that is related to the bacterial toxin Mcf (Makes caterpillar floppy) of the entomopathogen *Photorhabdus luminescens*, and the protein toxin was named Fit (*P. fluorescens* insecticidal toxin). Mcf of *P. luminescens* carries a Bcl2-homology 3 (BH3)-like domain, and *Escherichia coli* expressing *Mcf* infects the tobacco hornworm, *Manduca sexta*, and induces apoptosis in the midgut epithelium cells (Daborn *et al.*, 2002). The BH3-like domain of Mcf is conserved in the Fit toxin, suggesting that the toxin also promotes apoptosis in insects. When injected into the haemocoel, *P. protegens* killed the larvae of *P. sexta* and the greater wax moth, *Galleria mellonella*, and mutants

of Fit toxin genes were less virulent to the insects. As *P. protegens* is a rhizobacterium, it is unlikely that the bacterium encounters the caterpillars in nature. Further investigations are required to determine whether the plant-protecting *P. protegens* can also kill soil-borne insects or not.

Insect symbionts can counteract plant defences

Insect-associated microbes also alter the outcome of plant–insect interactions, and the mechanisms of such alterations often depend on their localization. Some insect symbionts reside within the insect body and counteract plant defence responses by increasing the production of enzymes that are required by insects. The mountain pine beetle, *Dendroctonus ponderosae*, colonizes and kills conifers in North America and is expanding its host tree range due to climate change (Raffa *et al.*, 2013). Pine trees synthesize terpenoids that are toxic to beetles. A recent metagenomic study revealed that *D. ponderosae* is strongly associated with microbial communities that are enriched with genes involved in terpene degradation compared with other plant biomass-processing microbial communities (Adams *et al.*, 2013). Furthermore, the bacteria associated with *D. ponderosae* were shown to metabolize monoterpenes and diterpene acids (Boone *et al.*, 2013). Thus, the microbial community of *D. ponderosae* seems to contribute to overcome the plant's terpenoid-based defences by degrading them (Boone *et al.*, 2013).

Gut-associated bacteria of the Western corn rootworm, *Diabrotica virgifera virgifera*, are reported to facilitate adaptation to non-host plants such as soybean (*Glycine max*), which is introduced into the corn field for crop rotation (Chu *et al.*, 2013). In this case, increased activity of a digestive enzyme (cysteine protease) of the Western corn rootworm is associated with the presence of gut microbiota. As expression of a cysteine protease inhibitor is a defence strategy of the plant against coleopteran insects, increased protease activity in the Western corn rootworm is speculated to be a counterdefence strategy of the insect (Curzi *et al.*, 2012). How gut-associated microbes increase the cysteine protease activity in the insect intestine is, however, not known. Similarly, the velvetbean caterpillar, *Anticarsia gemmatalis*, a soybean pest, is associated with gut bacteria that can be removed by tetracycline application. The activity of gut serine proteinases is suppressed by tetracycline application, indicating the involvement of bacterial symbionts in the protease production (Visotto *et al.*, 2009). Additionally, the gut bacteria isolated from the caterpillar express proteases (Pilon *et al.*, 2013). Thus, higher production of proteases induced or produced by the bacteria might contribute to the adaptation of the caterpillar to the soybean plant, which is rich in protease inhibitors (Visotto *et al.*, 2009).

Insect symbionts can interfere with plant defence signalling

Some bacteria exert their effects at the interface of plants and insects. Insect oral secretions (saliva and/or regurgitants) have direct contact with plant cells and can interfere

with plant cell biology. Insect oral secretions may contain insect symbionts and/or symbiont-derived proteins that interfere with the outcome of the interactions. For example, the Colorado potato beetle, *Leptinotarsa decemlineata*, harbours multiple bacterial symbionts in its oral secretion that can be transferred to the plant when it feeds. The flagellin derived from one of the symbiotic bacteria *Pseudomonas* sp. of the beetle was shown to induce SA signalling. By using negative cross-talk between the JA and SA signalling pathways, the flagellin suppresses JA signalling pathway, reducing resistance against the beetle. Thus, the beetle gains more weight when associated with the symbiotic bacteria (Chung *et al.*, 2013). *Wolbachia* sp. associated with the Western corn rootworm was also shown to suppress defence-related gene expression in maize plants, although the effect of defence gene suppression on insect performance was not evaluated (Barr *et al.*, 2010). It is noteworthy that contradictory results were reported recently (Robert *et al.*, 2013), suggesting that the observed effect of symbionts can be context dependent. In contrast to the positive effect of the beetle-associated *Pseudomonas* sp., the obligate symbiont of aphids, *B. aphidicola*, may trigger plant defence responses that act against host aphids. The chaperonin GroEL, which seems to be derived from *B. aphidicola*, was identified in the saliva of the potato aphid, *Macrosiphum euphorbiae*. Application of GroEL onto *Arabidopsis* induced the marker genes of pattern-triggered immunity, and delivery of GroEL into tomato (*Solanum lycopersicum*) also triggered this immunity and reduced the fecundity of the potato aphid on tomato (Chaudhary *et al.*, 2014). As *B. aphidicola* is an essential partner of aphids, it is not surprising to see that plants developed the means to recognize the *B. aphidicola*-derived protein in aphid saliva and trigger defence responses against the aphids. Although GroEL triggers plant defence responses, aphids can still feed on the plants, suggesting that aphids (or their symbionts) might have developed strategies, such as effectors, to overcome or suppress the GroEL-induced defence responses (Hogenhout and Bos, 2011; Chaudhary *et al.*, 2014). Further studies are required to test whether *B. aphidicola* GroEL triggers plant defence responses in other aphid–plant systems.

Insect symbionts can alter plant physiology and morphology

Some insect symbionts can indirectly enhance plant nutritional supply to the host insects at the interface of plants and insects. Several insects are known to alter source–sink relationships in plants and/or to prevent leaf tissues from senescing in order to meet their nutritional requirements and to buffer seasonal variations in nutrient provisioning (Kaiser *et al.*, 2010; Giron and Huguet, 2011). This has been clearly demonstrated in several galling insects and more recently in a leaf-miner system. More specifically, the herbivorous leaf-mining moth *Phyllonorycter blancardella* induces spectacular green and metabolically active areas in otherwise yellow senescent leaves, known as 'green islands', through a manipulation of the plant cytokinin (CK) profile (Giron *et al.*, 2007). This allows insects to manipulate the leaf protein–sugar content

in order to overcome a food supply that is highly variable and nutritionally suboptimal, particularly under senescing conditions (Body *et al.*, 2013). Interestingly, the presence of symbiotic bacteria is required for inducing the green-island phenotype and for the control of food nutritional imbalances (Kaiser *et al.*, 2010; Body *et al.*, 2013). Although CKs are phytohormones that promote plant growth and play a key role in leaf senescence and source–sink relationships, they also interfere with plant defence by promoting JA-mediated signalling (Erb *et al.*, 2012). This suggests that CK-mediated alterations of the plant by insects rely on a finely tuned and complex regulation where the specific quantity and composition of CKs produced, the nature of the target cells, the developmental state of the plant, and the hormonal balance between CKs and other phytohormones are key factors (Giron *et al.*, 2013). In this process, bacterial symbionts have been proved to be instrumental and may use similar strategies to plant-associated bacteria to manipulate the phytohormonal balance and gene expression pattern of their host plant causing metabolic and eventually morphological modifications (Giron and Glevarec, 2014).

Insect-borne plant pathogens can manipulate plants to attract vectors

Insect-associated microbes can be pathogenic to plants, and it is common for these plant pathogens to manipulate plant–insect interactions to enhance their own dissemination. Since plant pathogens can be injected and can propagate and spread systemically in plants, they can exert their effects on host plants for a long period of time. Some of these pathogens not only suppress plant defence systems but also change the plant architecture and/or physiology to lure vector insects to increase the chances of dispersal. Phytoplasmas are insect-transmitted plant pathogens and induce various symptoms, which include stunning morphological changes. Recent studies showed that phytoplasmas secrete effector proteins to induce such alterations. One of the effector proteins, SAP11, was identified in aster yellows phytoplasma strain Witches' Broom (AY-WB, *Candidatus* (*Ca*.) Phytoplasma asteris), which is transmitted by the leafhopper *Macrosteles quadrilineatus* (Bai *et al.*, 2009). SAP11 causes destabilization of a subset of *Arabidopsis* TCP transcription factors, which are part of the regulatory components of JA synthesis (Sugio *et al.*, 2011). As JA signalling induces *Arabidopsis* defence against leafhoppers, the latter produce more progeny on the phytoplasma infected- and SAP11-expressing *Arabidopsis* plants compared with non-infected wild-type plants. AY-WB also induces dramatic morphological changes in infected *Arabidopsis*, which includes the production of leaf-like flowers (phyllody and virescence) and many stems (witches' broom). SAP54 was identified as a phytoplasma effector that produces leaf-like flowers in *Arabidopsis*. SAP54 binds to members of the MADS-domain transcription family, which have a central role in the regulation of flower development, and mediates their degradation (Maclean *et al.*, 2014). Notably, *Arabidopsis* plants with leaf-like flowers induced by either the SAP54 single gene or AY-WB infection are more

attractive to the leafhopper vector, *M. quadrilineatus*, for oviposition (Maclean *et al.*, 2014). Thus, SAP54 allows the phytoplasma to alter the plant morphology, which in turn increases attraction of the leafhopper and possibly enhances the chance of its dissemination in nature. Interestingly, an *Arabidopsis ap1* mutant, which produces leaf-like flowers, is not more attractive to insect colonization over healthy plants. This result indicates that the flower morphology in *ap1* mutant is not sufficient to attract the insect and/or that SAP54 exerts its effect beyond the growth of leaf-like flowers, such as manipulation of the phytohormone-mediated defence system (Maclean *et al.*, 2014). Two effectors of AY-WB phytoplasma (SAP54 and SAP11) and an effector protein (TENGU) identified from the *Ca. P.* asteris OY strain all induce the production of many leaves and stems in *Arabidopsis* or *Nicotiana benthamiana* and create a bushy appearance, which is a characteristic symptom of phytoplasma infection (Hoshi *et al.*, 2009; Sugio *et al.*, 2011; Maclean *et al.*, 2014). A hypothesis that has not been tested yet is that such a bushy morphology caused by production of young tender tissues can be more attractive to insect vectors (Hogenhout *et al.*, 2008).

Some plant pathogens can also change the plant volatile composition to attract insect vectors. A phytoplasma strain, *Ca. P.* mali, changes the apple volatile composition and lures the psyllid vector *Cacopsylla picta* (Mayer *et al.*, 2008*a, b*). *Ca. P.* mali increases the emission of β-caryophyllene in apple tree, a sesquiterpene that attracts the psyllid. Similarly, *Ca.* Liberibacter asiaticus changes the volatile composition of infected plants to attract the psyllid vector *Diaphorina citri* and promote its dissemination. The Liberibacter infection induces the release of methyl salicylate, which *D. citri* is attracted to (Mann *et al.*, 2012). The mechanisms employed by these two pathogens to change the plant volatiles have not yet been reported.

Symbionts of insect vectors can interfere with transmission of plant pathogens

Some insect-associated bacteria may not be transferred from insect to plant but may interfere with the transmission of another insect-associated microbe. A chaperonin protein, GroEL, of the obligate symbiont of aphids, *Buchnera*, was shown to bind to the coat proteins of the luteovirus potato leafroll virus (Van den Heuvel *et al.*, 1994). The GroEL is produced by *Buchnera* in bacteriocytes and thought to be released into aphid haemolymph. Antibiotic treatment of the aphid reduced the amount of GroEL in haemolymph and reduced virus transmission by more than 70%. As the major coat protein of the virus was found to be degraded in the antibiotic-treated aphids, it was proposed that GroEL binds to and protects the virus from enzymatic breakdown in the aphid haemolymph and contributes to retention of the virus in the aphid and eventual transmission to the plant (Van den Heuvel *et al.*, 1994). However, contradictory results have been presented from a study of pea aphid and another luteovirus (barley yellow dwarf virus) interaction (Bouvaine *et al.*, 2011). In the case of another hemipteran insect,

whitefly, a secondary symbiont is reported to be involved in the transmission of the begomovirus tomato yellow leaf curl virus (TYLCV). While the Israeli whitefly B biotype, which is strongly associated with *Hamiltonella*, transmits the virus efficiently, the Q biotype, which is associated with other secondary bacterial symbionts, does not transmit the virus well (Gottlieb *et al.*, 2010). Studies have shown that GroEL of *Hamiltonella* interacts with the TYLCV coat protein *in vitro* and *in vivo* and contributes to virus transmission by the whitefly (Morin *et al.*, 1999; Gottlieb *et al.*, 2010), and that the interaction between GroEL and the TYLCV coat protein occurs only in the biotype that carries *Hamiltonella* (Gottlieb *et al.*, 2010). Whether insect-associated microbes alter the transmission rate of plant-pathogenic bacteria has not yet been reported. This is a key research area that requires further investigation to understand disease epidemiology and to improve disease control strategies.

Conclusion

Rapid advances in high-throughput sequencing technologies provide us with the tools to investigate community diversity and structure of plant- or insect-associated microbes, which are mostly uncultivable. In recent years, these technologies have highlighted a wide range of interactions between microbes and plant or insect hosts, from long-term and intimate relationships to more temporary or occasional interactions and involving different clades of bacteria. However, we are far from a complete characterization of plant and insect microbiomes and their variation influenced by environmental factors. Ecological webs indeed offer many opportunities for the acquisition, transmission, and selection of plant and insect microbes, via interactions either between them, through multitrophic interactions, or from other environmental sources (Zhu *et al.*, 2014). It is now clearly established that the acquisition of heritable or environmental microbial communities have strong ecological and evolutionary impacts for the host, enabling biological adaptations to new habitats and the development of novel responses to environmental stresses. We have shown in this review that the evolutionary trajectories of host-associated microbial communities are very diverse and highly complex, being shaped by multiple forces and mechanisms. This is reflected by the numerous types of relationships, from strict parasitism to obligate mutualism, the possibility of evolving mutualism from parasitism and vice versa, the occurrence of rapid shifts in bacterial communities, and eventually the exchange of genetic material between interacting organisms.

We have discussed here the mechanisms by which plant-associated bacteria can change the hormonal balance and physiology of host plants with profound consequences on plant–insect interactions at several trophic levels (Giron *et al.*, 2013; Pangesti *et al.*, 2013; Zhu *et al.*, 2014). We have also shown how microbial communities hosted by insect herbivores can manipulate plant primary or secondary metabolisms through a variety of mechanisms. Whether subsets of plant- and insect- associated bacteria use similar strategies to

interfere with plant physiology deserves further investigation (Giron & Glevarec, 2014). The underlying mechanisms of microbe–plant–insect interactions have only recently started to be understood. Recent studies have shown that as well as the classical defence hormones, SA, JA, and ET, various other growth-related hormones (CK, auxin, and brassinosteroids) are also involved in fine-tuning these microbe–plant–insect interactions (Erb *et al.*, 2012; Meldau *et al.*, 2012; Giron *et al.*, 2013). Pioneering studies also indicate that effector proteins secreted from bacteria or insects are one of the keys to understanding the mechanisms of the interactions. Further studies will reveal new mechanisms by which insect- or plant-associated microbes interfere with plant–insect interactions. Understanding such mechanisms in model systems under controlled environments but also in more natural ecological settings will help to shed light on the evolution and functioning of complex multitrophic interactions in which plants, herbivorous insects, and micro-organisms are inserted.

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