

Minireview

The secret life of plant-beneficial rhizosphere bacteria: insects as alternative hosts

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Summary

Root-colonizing bacteria have been intensively investigated for their intimate relationship with plants and their manifold plant-beneficial activities. They can inhibit growth and activity of pathogens or induce defence responses. In recent years, evidence has emerged that several plant-beneficial rhizosphere bacteria do not only associate with plants but also with insects. Their relationships with insects range from pathogenic to mutualistic and some rhizobacteria can use insects as vectors for dispersal to new host plants. Thus, the interactions of these bacteria with their environment are even more complex than previously thought and can extend far beyond the rhizosphere. The discovery of this secret life of rhizobacteria represents an exciting new field of research that should link the fields of plant-microbe and insect-microbe interactions. In this review, we provide examples of plant-beneficial rhizosphere bacteria that use insects as alternative hosts, and of potentially rhizosphere-competent insect

sympionts. We discuss the bacterial traits that may enable a host-switch between plants and insects and further set the multi-host lifestyle of rhizobacteria into an evolutionary and ecological context. Finally, we identify important open research questions and discuss perspectives on the use of these rhizobacteria in agriculture.

Introduction

Many higher organisms co-occur in the same habitat and can interact with each other, providing opportunities for the exchange of microbes between, for example, plants and animals. This exchange can have significant ecological implications but has been little explored.

Over the past decades, it has become clear that the plant rhizosphere microbiome is of great importance for plant health (Berendsen *et al.*, 2012; Mendes *et al.*, 2013; Berg *et al.*, 2017; Trivedi *et al.*, 2020). Rhizosphere microbes can be present in the soil directly around the root, on the root surface, or inside the plant root. They are mostly recruited from bulk soil by plant root exudates (Sasse *et al.*, 2018), and can potentially be pathogenic but also beneficial to the plant. Within the bacterial community of the rhizosphere, many individual members as well as consortia of different species have been identified as plant-beneficial (Berendsen *et al.*, 2012; Berendsen *et al.*, 2018). They can suppress plant diseases by directly inhibiting pathogens, or indirectly by inducing defence responses in the plant (Pieterse *et al.*, 2014), and some can also promote plant growth in the absence of pathogens (Compant *et al.*, 2010). This makes these bacteria interesting candidates for the biological control of crop diseases or as biological fertilizers.

Plants do not only interact with microbes but also with higher organisms, including insects. Positive interactions with insects such as pollinators and insects that spread plant seeds are essential for plant reproduction. In contrast, herbivorous insects present a constant threat for plant growth and survival. Moreover, many of these

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insects are important vectors of viral and bacterial plant pathogens (Kluth *et al.*, 2002). Parasitoids and predatory insects may function as bodyguards of the plant by keeping these vectors and pest insects at bay (van Lenteren and Manzaroli, 1999). Like plants, insects harbour microbiomes that include beneficial bacteria and fungi that provide nutrients or protection against pathogens, parasites and predators (Douglas, 2015). They may also contain microorganisms with entomopathogenic activities. These could serve as a source for biological control agents to control pest insects, but they may also threaten crop yields in the case of pollinator pathogens.

The microbiomes of plants and insects have mostly been studied separately from each other, but since plant–insect interactions are so ubiquitous, it is not surprising that interactions between plant-associated bacteria and insects, and between insect-associated bacteria and plants have been observed. Recent studies (Allard *et al.*, 2018; Rebolleda Gómez and Ashman, 2019) suggest that pollinators can alter microbial communities of flowers, and it is even suggested that these communities can be used as an indicator of which pollinators visited the plant (Ushio *et al.*, 2015). These studies show that bacteria can be transferred from insects to plants, but they did not examine the function of these bacteria in both insects and plants.

Most examples of interactions between plant-associated bacteria and insects have been reported for pathogens and pest insects, especially those inhabiting above-ground parts of the plant. The dispersal of plant pathogens by insect vectors is a common phenomenon (Orlovskis *et al.*, 2015). For example, the bacteria that cause the Huanglongbing disease of citrus are spread from plant to plant by a phloem-feeding insect (da Graça *et al.*, 2016). A review by Nadarasah and Stavrinides (2011) highlights that insects can also serve as a host for plant pathogenic bacteria. They state that some plant pathogens may originate from entomopathogens, suggesting that plants are not their original habitat. Diminished insect virulence may facilitate the vectoring role of insects in spreading the pathogen to its plant host. For plant pathogenic bacteria, frequent encounters with insects that share their habitat have resulted in the evolution of mechanisms that allow colonization of two different hosts. A loss of virulence or increased virulence towards one or both hosts could change the nature of the symbioses over time, for example from entomopathogenicity to commensalism, or even insect mutualism.

Plant-beneficial rhizosphere bacteria can influence plant–insect interactions by activating defence responses in plants or by changing volatile compound emissions, and therefore attractiveness, of the plant (Pineda

et al., 2013, 2017; Pangesti *et al.*, 2015). In turn, in response to insect herbivory, plants can alter their microbiome to increase resistance to herbivory for the following generation (Kong *et al.*, 2016; Pineda *et al.*, 2017).

Even though many insects live in and on the soil, close to plant roots, direct interactions between insects and rhizosphere bacteria have received little attention. In the late 90s, experiments in controlled microcosms suggested that a *Pseudomonas chlororaphis* strain could be dispersed by insects (Snyder *et al.*, 1998, 1999). Almost a decade later, the discovery that certain plant-beneficial *Pseudomonas* strains produce insecticidal compounds that are highly toxic to a range of pest insects (Péchy-Tarr *et al.*, 2008) triggered investigations on insects as alternative hosts for these bacteria. Recently, more evidence emerged that plant-beneficial rhizosphere bacteria can use insects as an alternative host and dispersal vector (Flury *et al.*, 2019; Kim *et al.*, 2019). Perhaps this phenomenon is as common for plant-beneficial rhizosphere bacteria as it is for foliar plant-pathogenic bacteria.

In this review, we discuss recently discovered examples of rhizosphere bacteria with an alternative insect-associated lifestyle, bacterial traits that facilitate this multi-host lifestyle, how such a lifestyle may evolve, and lastly the ecological and agricultural implications.

Insects as vectors and alternative hosts for rhizosphere bacteria

For plant-pathogenic bacteria, it is well-known that they can be vectored by insects (Nadarasah and Stavrinides, 2011; Orlovskis *et al.*, 2015). Like plant pathogens, rhizosphere bacteria frequently encounter insects with which they share their habitat. Lately, evidence is rising that certain plant-beneficial rhizosphere bacteria can successfully colonize insects and use them as a means of dispersal to the rhizosphere of new host plants. In the following, we discuss two recently published cases of this phenomenon.

Plant-beneficial Pseudomonas protegens and chlororaphis – opportunistic pathogens of insects?

The genus *Pseudomonas* is well-known for its plant-beneficial rhizosphere species and their role in disease-suppressive soils and plant growth promotion (Haas and Défago, 2005; Mercado-Blanco and Bakker, 2007; Kupferschmied *et al.*, 2013; Almario *et al.*, 2014; Thomashow *et al.*, 2019). Among this group of rhizosphere bacteria, *Pseudomonas protegens* strains such as CHA0 and Pf-5 can protect plants against various root pathogens (Haas and Défago, 2005; Kupferschmied

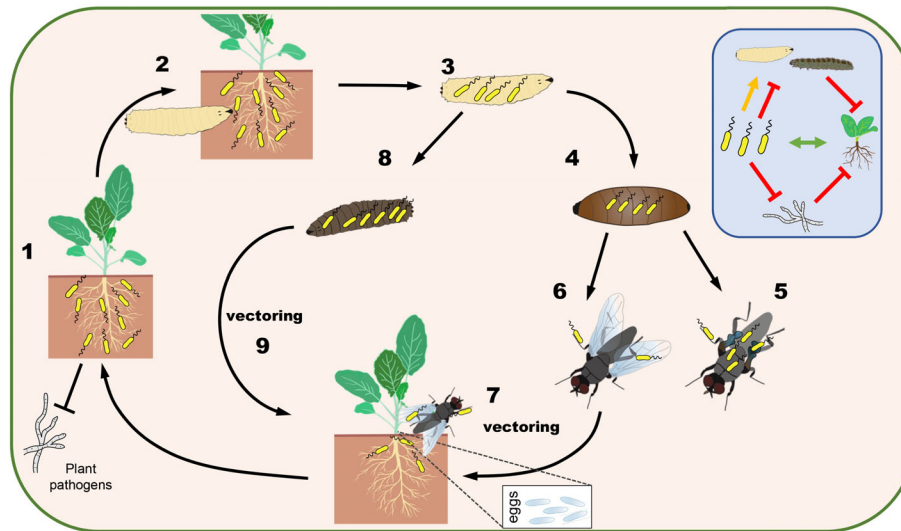


Fig. 1. *Pseudomonas protegens* has a multi-host lifestyle in plants and insects and can be vectored to new plants by the cabbage root fly *Delia radicum*. 1. The rhizosphere is colonized by *P. protegens*, which protects the plant against pathogenic fungi. 2. Insect larvae that feed on the roots of the plant ingest *P. protegens* cells. 3. *Pseudomonas protegens* persists in larvae of the cabbage root fly (*D. radicum*) and other insect larvae. 4. *Pseudomonas protegens* persists in *D. radicum* pupae. 5. The pupae develop into deformed adults or 6. into healthy flies. 7. Healthy *D. radicum* flies lay their eggs at the base of the plant on the soil and can transfer *P. protegens* cells to the rhizosphere. 8. When *P. protegens* kills the insect host, it mass replicates inside the larvae. 9. *Pseudomonas protegens* cells escape from the dead larvae, and migrate into the soil, from where they colonize the rhizosphere. **Inlay:** Diagram that indicates the nature of the interactions of the bacteria with the insect, the plant and soilborne plant pathogens. Green arrow, beneficial; orange arrow, neutral; red inhibition arrow, negative interaction. The figure has been adapted from Flury *et al.*, 2019.

et al., 2013) (Fig. 1) and can also induce systemic resistance against foliar diseases (Maurhofer *et al.*, 1994; Iavicoli *et al.*, 2003). Interestingly, both *P. protegens* strains also display an alternative lifestyle in insects. They produce the Fit toxin, an insecticidal protein that is closely related to the *Makes caterpillars floppy* (Mcf) toxin of *Photorhabdus* and *Xenorhabdus* species, both symbionts of entomopathogenic nematodes. *Pseudomonas protegens* strains kill insect larvae not only when injected into the insect hemocoel (Péchy-Tarr *et al.*, 2008), but also when fed to larvae of several lepidopteran and dipteran species (Olcott *et al.*, 2010; Ruffner *et al.*, 2013; Loper *et al.*, 2016; Rangel *et al.*, 2016; Ruiu and Mura, 2021) (Fig. 1). Within the *P. fluorescens* group only the species *P. protegens* and its close relative *Pseudomonas chlororaphis* exhibit strong insecticidal activity (Ruffner *et al.*, 2015; Flury *et al.*, 2016; Loper *et al.*, 2016), clearly distinguishing them from other plant-beneficial pseudomonads.

Many factors contributing to the insecticidal activity of *P. protegens* and *P. chlororaphis* strains have been identified over the last years (Péchy-Tarr *et al.*, 2008; Flury *et al.*, 2016, 2017; Keel, 2016; Kupferschmied *et al.*, 2016; Loper *et al.*, 2016; Rangel *et al.*, 2016; Vacheron *et al.*, 2019; Vesga *et al.*, 2020). Among them are antimicrobial metabolites, i.e. cyclic lipopeptides, rhizoxin and hydrogen cyanide, which are involved in pathogen suppression and insecticidal activity (Flury *et al.*, 2017), but also factors that are specifically

expressed inside insects (Péchy-Tarr *et al.*, 2013; Kupferschmied *et al.*, 2014; Flury *et al.*, 2017; Vesga *et al.*, 2020), including the Fit toxin, a chitinase, and a two-partner secretion system. Strain CHA0 has been shown to use these virulence factors and a type VI secretion device with associated toxic effectors to compete with the native insect gut microflora (Vacheron *et al.*, 2019) and to overcome the gut epithelial barrier (Vesga *et al.*, 2020). If the strain reaches the hemocoel it multiplies to very high numbers leading to fatal septicemia. The bacteria then use the insect cadaver as a vessel for mass reproduction, and the billions of resulting bacteria can spread and recolonize plant roots (Fig. 1).

The ecological relevance of the insect-associated lifestyle of these pseudomonads is not fully understood yet. For *P. protegens* lethal oral infections are mainly observed in experiments with laboratory-reared insect larvae fed on leaves or an artificial diet inoculated with the bacterium. The susceptible insects were either leaf-feeding lepidopteran species or dipteran species such as *Drosophila melanogaster* or *Musca domestica*, which normally feed on fruits or rotting organic material respectively (Péchy-Tarr *et al.*, 2008, 2013; Olcott *et al.*, 2010; Ruffner *et al.*, 2013; Flury *et al.*, 2016; Loper *et al.*, 2016; Rangel *et al.*, 2016; Ruiu and Mura, 2021). However, *P. protegens* had no or very little effect on the survival of root-feeding dipteran and coleopteran pests (Chiriboga *et al.*, 2018; Flury *et al.*, 2019; Jaffuel *et al.*, 2019). This might be due to the different physiology of these insect

groups, due to lower bacterial dosages taken up when larvae feed on colonized roots, or due to co-evolution of root-feeding insects with rhizobacteria towards an attenuated virulence. The latter would represent an advantage for pseudomonads if the survival of the insects enables the transportation of the bacteria to new root habitats.

Indeed, CHA0 can persist in *Delicia radicum* and *Otiorynchus sulcatus* throughout different developmental stages (Flury *et al.*, 2019) until the imago (Fig. 1). This is of significant ecological impact, since adult insects cover larger distances, especially when in search of new host plants for egg deposition. In fact, CHA0 ingested by larvae of *D. radicum* can be vectored by the resulting adults from the roots of one plant to the roots of another plant under experimental conditions (Flury *et al.*, 2019) (Fig. 1). Already in the 90s a study by Snyder and colleagues showed that *P. chlororaphis* strain L11, a strong root-colonizer with the ability to move into the foliage, can persist from the larval to the adult stage in the southern corn rootworm, *Diabrotica undecimpunctata* subsp. *howardi*, and can be vectored between corn plants (Snyder *et al.*, 1998). While nothing is known about the biocontrol ability of L11 and the study has received little attention for a long time, this finding now strongly supports the hypothesis that *P. protegens* and related strains can use insects as means of dispersal.

For many years, interactions with insects had only been studied for plant-derived *P. protegens* and *P. chlororaphis* strains. However, a recent isolation effort from soil organisms revealed that both species are also naturally associated with healthy coleopteran insects and myriapods (Vesga *et al.*, 2021). Like root isolates, the strains isolated from insects have both lepidopteran killing and plant disease suppressive abilities (Vesga *et al.*, 2021). This supports the hypothesis that insects are alternative hosts for these bacteria in natural ecosystems.

In summary, *P. protegens* and *P. chlororaphis* can adopt contrasting lifestyles as effective colonizers of plant roots and insects. The same strains possess the tools enabling them to colonize both hosts and likely to switch between them. While the interaction of the bacteria with plants is beneficial for both partners, for the insects it can be commensal or pathogenic (Fig. 1). They probably use the insect as vector for dispersal or as a vessel for survival and multiplication.

Mutualistic interactions between *Streptomyces globisporus* and pollinators

While *P. protegens* is either pathogenic or commensal to its insect hosts, a *Streptomyces globisporus* strain that is almost identical to a plant-beneficial *Streptomyces*

sp. found in the rhizosphere can engage in a mutualistic interaction with insects (Kim *et al.*, 2019). *Streptomyces* is a genus of Gram-positive filamentous bacteria that can be found in a wide variety of habitats ranging from soils (Delgado-Baquerizo *et al.*, 2018) to insects (Kaltenpoth, 2009) to humans (Kapadia *et al.*, 2007). While some species are plant pathogens (Loria *et al.*, 1997), other species of *Streptomyces* are associated with disease-suppressive soils (Cha *et al.*, 2016), and there is evidence that they can improve plant growth (Jog *et al.*, 2014). Additionally, *Streptomyces* are known to produce volatile organic compounds (VOCs), some of which are used to interact with arthropods (Weisskopf *et al.*, 2021).

Streptomyces globisporus strain SP6C4, isolated from strawberry flowers, protects strawberry flowers and fruits against the fungal pathogen *Botrytis cinerea* (Kim *et al.*, 2019). Interestingly, the SP6C4 genome is nearly identical (99.99% sequence identity) to the *Fusarium*-suppressive strain S4-7 isolated from strawberry field soil (Cha *et al.*, 2016). Moreover, SP6C4 can endophytically migrate from the root to the stem as well as from the flower to the stem (Kim *et al.*, 2019) (Fig. 2). This indicates that migration from the root to the flower or vice versa might be possible.

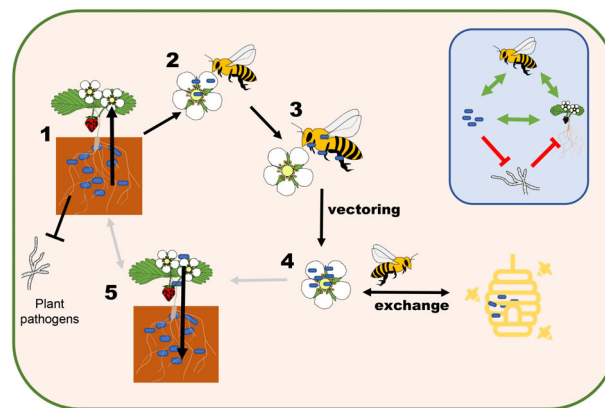


Fig. 2. *Streptomyces globisporus* SP6C4 has a multi-host lifestyle colonizing strawberry plants and honeybees, which can serve as vectors for dispersal. 1. In the rhizosphere, *S. globisporus* SP6C4 protects the plant from soil-borne diseases. 2. SP6C4 can potentially migrate from the rhizosphere to the flower. The bacteria protect the flower from fungal infection. Honeybees pollinate the plant and pick up the bacteria externally and by ingestion. 3. The bacteria protect honeybees from entomopathogens. The honeybee spreads the bacteria to new flowers while pollinating. 4. The honeybee facilitates exchange between the beehive and strawberry flowers. 5. *Streptomyces globisporus* migrates from the flowers to the rhizosphere. Grey arrows indicate speculative interactions for which the definite evidence is still lacking. **Inlay:** Diagram that indicates the nature of the interactions of the bacteria with the insect, the plant and soil-borne pathogens. Green arrow, positive; red inhibition arrow, negative interactions.

In an experimental setup, honeybees could transmit strain SP6C4 between strawberry flowers and from the beehive to strawberry flowers (Kim *et al.*, 2019) (Fig. 2). Flowers that were 'inoculated' by the bees in this experiment showed a decrease of disease symptoms caused by *B. cinerea* (Kim *et al.*, 2019). Future experiments should point out whether strain SP6C4 can migrate to the roots and if it can provide disease suppression against *Fusarium oxysporum*. Additionally, it would be interesting to know if strain SP6C4 inoculated in the soil will migrate to strawberry flowers, where it could be vectored by honeybees (Fig. 2).

An earlier 16S rRNA gene-based microbial community profiling study points towards a similar function of *Streptomyces* in tomato plants and their bumblebee pollinators (Kwon *et al.*, 2018). The bacterial communities of tomato flowers and bumblebee hives in the same greenhouse became more similar over time, indicating microbe exchange between bumblebees and tomato flowers. Furthermore, *S. globisporus* consistently showed up in both communities with increasing relative abundance over time. Similar to the results from strawberries, a *Streptomyces* strain isolated from tomato flowers inhibited the growth of the necrotrophic fungus *B. cinerea* in an *in vitro* assay.

These studies show that plants can benefit from insect-mediated dispersal of beneficial *Streptomyces* bacteria. In turn, the bacteria, which can colonize below-ground and above-ground plant organs as well as insects, presumably profit from the plant host by obtaining nutrients and from the insect host by an increased range of dispersal, and perhaps also nutrient acquisition. But is there also benefit for the insect? An experiment with bumblebees showed that SP6C4 does not only antagonize plant pathogens but also the insect pathogens *Serratia marcescens* and *Paenibacillus larvae*, the causative agent of foulbrood disease (Kim *et al.*, 2019). Recently, other *Streptomyces* strains isolated from pollen in beehives were also shown to protect honeybees against *P. larvae* (Grubbs *et al.*, 2021). Thus, by dispersing SP6C4, honeybees may be rewarded with healthier flowers and protection against entomopathogens. This means that all three actors profit from the interaction and natural selection might favour this multipartite mutualism (Fig. 2). Interestingly, a recent study implicates that honeybee behaviour is affected by geosmin, a VOC produced by numerous *Streptomyces* species (Scarano *et al.*, 2021). Low concentrations of geosmin strongly suppress the defensive behaviour of bees. The implications of this behavioural change in bees are unknown, but geosmin produced by *Streptomyces coelicolor* attracts springtails, which then feed on the bacterial colonies and disperse *S. coelicolor* spores (Becher *et al.*, 2020). VOCs may also be

important for the interaction between *S. globisporus* and honeybees and could be a topic of future research.

The examples of *P. protegens* and *S. globisporus* demonstrate that insects can play an important role as alternative hosts and for the dispersal of plant-beneficial rhizosphere bacteria and point out that multi-host lifestyles including plant and insect partners might be much more common than known to date and are thus an intriguing field for future research.

Identifying bacteria with both rhizosphere- and insect-associated lifestyles

Plant-beneficial bacteria with a multi-host lifestyle in both plants and insects have neither received broad attention from the field of plant–microbe interactions nor from the field of microbe–insect interactions, but when examining the literature from these fields more closely there are indications that such bacteria may be more common than previously assumed. We will discuss examples of bacterial taxa that potentially switch between rhizosphere- and insect-associated lifestyles, but for which conclusive evidence is not available yet. We focus on bacteria that are mutualistic to the plant and mutualistic or pathogenic to the insect.

Mutualistic interactions between plant-beneficial Burkholderia spp. and stinkbugs

Members of the genus *Burkholderia* are typically found in the rhizosphere and often show plant-beneficial activities (Elliott *et al.*, 2007; de los Santos-Villalobos *et al.*, 2012; Paungfoo-Lonhienne *et al.*, 2016). Several stinkbug families, which can be important agricultural pests, harbour obligate symbiotic *Burkholderia* bacteria in specialized midgut crypts (Kikuchi *et al.*, 2005, 2011). Studies of the stinkbug *Riptortus pedestris* and its symbiont *Burkholderia insecticola* have provided important insights into the stinkbug–*Burkholderia* symbiosis. Presumably, the bacteria provide a nutritional benefit to their host, resulting in increased growth rates and body mass (Kikuchi *et al.*, 2007). Stinkbugs do not vertically transmit their symbionts, but their nymphs acquire them *de novo* from the soil every generation (Kikuchi *et al.*, 2007). To overcome the risk of co-infection with non-symbiotic and even pathogenic bacteria, stinkbugs possess a tiny organ that serves as a selective filter that only allows their *Burkholderia* symbionts into the midgut crypts (Ohbayashi *et al.*, 2015). In *R. pedestris*, colonization by *B. insecticola* triggers closure of the midgut crypts, so that no other bacteria can enter (Kikuchi *et al.*, 2020). This adaptation allows the stinkbugs to reliably acquire the correct symbiont from the environment.

Many of the stinkbug-associated *Burkholderia* species fall into a distinct 'stinkbug-associated and environmental' (SBE) phylogenetic clade, but symbionts of the stinkbug suborders Largidae and Blissidae fall into the 'plant-associated beneficial and environmental (PBE)' clade of *Burkholderia* species that also harbours many beneficial rhizosphere bacteria (Takeshita *et al.*, 2015; Gordon *et al.*, 2016). Several PBE species belonging to the genus *Burkholderia* and the closely related genus *Pandoraea* that were isolated from the soil or rhizosphere and were fed to symbiont-free stinkbug nymphs successfully established a symbiosis with the stinkbug. They were only slightly less effective than SBE species (Itoh *et al.*, 2019). It is remarkable that free-living and plant-associated bacteria can colonize such a selective and specialized symbiont sorting organ, and it suggests that the mechanisms needed for establishing the symbiosis with stinkbugs were present in the ancestor of the genera *Burkholderia* and *Pandoraea*. These mechanisms may also be important for their free-living and plant-associated lifestyle. In fact, there is evidence that some SBE species also have a free-living and possibly plant-associated lifestyle. The SBE symbiont *B. insecticola* of the soybean-feeding stinkbug *R. pedestris* (syn. *R. clavatus*) was found on soybean roots, and newly hatched nymphs acquired their symbionts from soybeans potted in field soil (Kikuchi *et al.*, 2007). Future experiments could point out whether the SBE species and the stinkbug symbionts in the PBE clade can also colonize the rhizosphere, and perhaps even have plant-beneficial properties.

Bacillus thuringiensis: insect pathogen and plant benefactor?

Bacillus thuringiensis is well-known for its production of insecticidal toxins. Because of this feature, it is used as a biocontrol organism to ward off pest insects in crops. Most research has focused on the biocontrol properties of *B. thuringiensis*, while its ecology has received less attention. *Bacillus thuringiensis* has not only been found in insects, but in many different environments such as soils, rhizospheres and plant endospheres, and is possibly vectored between these environments by nematodes (Ruan *et al.*, 2015). *Bacillus thuringiensis* was found to be naturally present in different plant compartments of cotton in fields that had never been treated with *B. thuringiensis* products before (Monnerat *et al.*, 2009). Moreover, when added to soil close to cotton roots, *B. thuringiensis* translocated from the soil to the roots and moved endophytically to the leaves, which then became toxic to two Lepidoptera species (Monnerat *et al.*, 2009). Further research should point out whether *B. thuringiensis* naturally present in (agro)ecosystems can help plants to ward off insects similar to when it is

artificially introduced as a biocontrol agent. *Bacillus thuringiensis* also has other plant-beneficial properties. Certain *B. thuringiensis* strains produce compounds that can promote plant growth or suppress plant pathogens (Azizoglu, 2019). However, it is unknown whether these plant-beneficial strains produce insecticidal toxins and whether insecticidal *B. thuringiensis* strains are plant-beneficial. The identification of *B. thuringiensis* strains with both insecticidal and plant-beneficial activities would be highly interesting and could lead to the development of 2-in-1 products serving as biopesticides and biofertilizers.

The rhizosphere as alternative habitat for the nematode symbiont and insect pathogen Photorhabdus luminescens

Photorhabdus bacteria live in symbiosis with entomopathogenic nematodes. The nematode enters the insect hemolymph and regurgitates the *Photorhabdus* bacteria, which produce diverse toxins and cause a lethal septicemia in the insect host (Waterfield *et al.*, 2009). *Photorhabdus luminescens* exists in two phenotypically different, but genetically identical forms. The so-called primary cells are living in symbiosis with the nematodes. Upon prolonged cultivation, but also in the insect host, primary cells can convert into secondary cells (Eckstein and Heermann, 2019). These secondary cells do not re-associate with nematodes, and compared to primary cells they exhibit transcriptional differences, which indicate adaptations to a life outside the insect host (Eckstein *et al.*, 2019). Secondary cells exhibit features similar to known plant-beneficial rhizosphere bacteria. Unlike primary cells, they exhibit chemotaxis towards root exudates and can colonize *Arabidopsis thaliana* roots (Regaiolo *et al.*, 2020). On the roots, *P. luminescens* might exhibit plant-beneficial traits. In plate inhibition assays, secondary cells inhibited growth of the plant pathogen *Fusarium graminearum* (Regaiolo *et al.*, 2020). If secondary cells retained insecticidal activity, they could potentially protect the roots against herbivores. In summary, these findings indicate that *P. luminescens* may exhibit, in addition to its insect and nematode associated lifestyles, adaptations to living in the rhizosphere. Therefore, it would be of great interest to reveal whether secondary *Photorhabdus* cells are indeed associated with roots in natural contexts.

Is the beewolf symbiont Streptomyces philanthi rhizosphere competent?

Beewolf wasps rear a symbiont, *Streptomyces philanthi*, in their antennal glands, which they secrete and smear on their honeybee prey and on their brood cells to protect

their developing offspring from fungal infections (Strohm and Eduard Linsenmair, 2001; Kaltenpoth *et al.*, 2005, 2006). This form of vertical symbiont transmission (see Box 1) ensures that the larvae ingest the symbiont when hatching, and later incorporate the symbiont into their cocoon, from which the emerging adult reacquires the symbiont. Interestingly, a *Streptomyces* isolate from the rhizosphere of chili pepper was identified as *S. philanthi*, based on 100% sequence similarity of a partial sequence of the 16S rRNA gene and it was able to control various plant diseases (Boukaew *et al.*, 2011). Although it is not sure whether this strain is also a beewolf symbiont, the close relatedness to the beewolf-associated *S. philanthi* indicates that there might be an overlap of traits that allow *S. philanthi* to establish a symbiosis with insects and with plants.

Could beewolf wasps possibly acquire an environmental plant-beneficial *Streptomyces* symbiont? Beewolf wasps burrow in the soil where they stash their prey (honeybees) and lay their eggs. The emerging larvae live and pupate in the soil. Beewolves that dig their burrows close to plants may occasionally pick up rhizosphere

bacteria in their antennae. However, beewolves have evolved a mechanism that blocks the antennal gland secretions in the antennal segments that contain opportunistic bacteria, which prevents their further spreading (Nechitaylo *et al.*, 2014). This is similar to how stinkbugs close off their symbiont-housing organs (Kikuchi *et al.*, 2020). Nevertheless, opportunistic environmental Actinobacteria were sometimes found in the antennae in high densities (Nechitaylo *et al.*, 2014). Contact between rhizosphere bacteria and the beewolf's antenna is likely, and one can hypothesize that if the bacterium is very closely related to the beewolf symbiont, and shares traits necessary for survival in the glands, a symbiosis could be established. This remains purely speculative but invites taking a closer look, starting with testing the ability of the rhizosphere isolate to establish a symbiosis with a beewolf.

Bacterial traits that facilitate a multi-host lifestyle

Many rhizosphere bacteria are known for their metabolic diversity, and they can produce and release a wide range of secondary metabolites (Lucke *et al.*, 2020). This allows them to cope with frequently changing conditions and competition from other microbes. The bacteria discussed in the examples above belong to genera such as *Pseudomonas*, *Burkholderia*, *Streptomyces* or *Bacillus*. Representatives of these genera can be found in diverse environments and are associated to different hosts ranging from plants to insects to mammals, for which they can be beneficial or pathogenic (Vial *et al.*, 2007; Silby *et al.*, 2011; Seipke *et al.*, 2012; Patiño-Navarrete and Sanchis, 2017). Many species are free-living with large genomes, and the wide metabolic range associated with these large genomes is presumably what makes these bacteria so versatile.

We have discussed examples of bacterial species or strains that can successfully switch from an insect host to a plant or vice versa. These bacteria are beneficial to plants, and they can be parasitic, commensal, or mutualistic to insects. They may have undergone host-specific adaptations, such as the acquirement and/or adaptation of genes enabling the production of the insecticidal Fit toxin of *P. protegens* and its host-specific regulation (Péchy-Tarr *et al.*, 2013; Ruffner *et al.*, 2013; Kupferschmied *et al.*, 2014; Flury *et al.*, 2016; Vesga *et al.*, 2021), but other bacterial traits may be involved in interactions with both plants and insects. Perhaps these bacteria use components of the same genetic toolbox for colonizing very different environments. Indeed, the obligate stinkbug symbiont *B. insecticola* can be replaced with related rhizosphere species, which provide similar benefits to the stinkbug (Hosokawa *et al.*, 2016). Likewise, multiple bacterial species, including *Pseudomonas*

Box 1. Horizontal versus vertical symbiont transmission in insects.

Most reported insect symbionts are vertically transmitted obligate mutualists (Salem *et al.*, 2015), whereby the symbionts are directly transmitted from the parent to the offspring. Horizontal transmission, whereby the symbiont is acquired *de novo* from the environment each generation, is less common in insects. Nevertheless, part of the insect gut microbiome is acquired from the environment through nutrition. The following table shows the characteristics of both types of symbiont transmission.

Horizontal transmission	Vertical transmission
<ul style="list-style-type: none"> • Offspring acquires symbiont from environment each generation • Symbiont may show little signs of coevolution: <ul style="list-style-type: none"> - Large genome - Can survive outside host - Incongruent phylogenies of host and symbiont • Host can show signs of coevolution: <ul style="list-style-type: none"> - Specialized symbiont-selecting organs, e.g. the constricted midgut region in stinkbugs (Ohbayashi <i>et al.</i>, 2015) 	<ul style="list-style-type: none"> • Parents directly transmit their symbionts to their offspring (e.g. via egg-smearing) • Symbionts show signs of coevolution: <ul style="list-style-type: none"> - Genome reduction - Cannot survive outside host - Congruent phylogenies of host and symbiont • Host can show signs of coevolution: <ul style="list-style-type: none"> - Symbiont-housing organs (Kaltenpoth <i>et al.</i>, 2006)

spp. and *Acinetobacter* spp., that were isolated from insects were shown to have functions that make them plant-growth promoting (Indiragandhi *et al.*, 2008; Vallet-Gely *et al.*, 2010; Vesga *et al.*, 2021). In this section, we describe bacterial traits and mechanisms that facilitate a lifestyle in both the rhizosphere and insects.

Flagella and lipopolysaccharides

Because plants and insects represent very different environments, bacteria may have evolved different colonization strategies for each host. However, there is evidence that some mechanisms are important for colonization of both plants and insects. For example, *B. insecticola* requires flagellar motility for the passage of the constricted gut region in the stinkbug midgut (Ohbayashi *et al.*, 2015). After passing through this region and before entering the midgut crypts, motility-related genes are downregulated under the influence of stress-related molecules from the insect, causing the bacteria to lose their flagellar motility (Ohbayashi *et al.*, 2019). Rhizosphere bacteria also need flagella to approach and colonize the plant roots (de Weger *et al.*, 1987), after which other proteins such as LapA are necessary for attachment to the root surface (Hinsa *et al.*, 2003). Additionally, research on plant-beneficial *Burkholderia* species shows the downregulation of flagellar motility once inside the host plant, similar to what happens in insects (Paungfoo-Lonhienne *et al.*, 2016). Likewise, in *P. protegens*, motor activity-related genes are expressed during the colonization of both the rhizosphere and lepidopteran insects (Vesga *et al.*, 2020).

Lipopolysaccharides (LPS) found on the outer cell membrane of Gram-negative bacteria play a role in virulence in many different host-pathogen systems. The O-antigen polysaccharide is required for the initial colonization of the *R. pedestris* gut by its *Burkholderia* symbiont (Kim *et al.*, 2016). Once established in the midgut, the symbionts lack the O-antigen, but they need the core oligosaccharide for maintenance of the symbiosis (Kim *et al.*, 2017). There is evidence that LPS components, including O-antigen, are also important for the colonization of roots by rhizosphere bacteria (de Weger *et al.*, 1989; Ormeño-Orrillo *et al.*, 2008; Li *et al.*, 2021b). In *P. protegens* CHA0, biosynthetic gene clusters for multiple O-antigen decorations exist (Kupferschmied *et al.*, 2016), which may reflect an adaptation to colonize different hosts. This is supported by a recent study showing that in CHA0 some O-antigenic polysaccharide gene clusters are expressed during root and insect colonization, while others are expressed specifically in insect backgrounds (Vesga *et al.*, 2020). CHA0 mutants lacking a specific O-antigen are no longer resistant to antimicrobial peptides that are a part of the insect immune

response and are significantly less virulent to insects (Kupferschmied *et al.*, 2016). These specific O-antigens are thus likely involved in avoiding recognition by the insect immune response.

Siderophores

Siderophores are a class of compounds that chelate iron for uptake from the environment and are commonly produced by fluorescent pseudomonads in the rhizosphere (Höfte and Bakker, 2007; Loper *et al.*, 2012; Zboralski and Filion, 2020). Siderophores play an important ecological role in the rhizosphere. Several rhizosphere bacteria can cross-utilize siderophores produced by others, providing a competitive advantage (Joshi *et al.*, 2006). This cross-utilization may directly inhibit pathogen growth (de los Santos-Villalobos *et al.*, 2012), but siderophores are also known to induce systemic resistance in plants (De Vleeschauwer *et al.*, 2008). Siderophore cross-utilization is also reported for siderophore-producing insect gut bacteria, which protect the diamondback moth against entomopathogens (Indiragandhi *et al.*, 2008). Additionally, 79% of the gut bacteria of the grasshopper *Sathrophyllia femorata* produced siderophores *in vitro* (Sonawane *et al.*, 2018). Siderophores are also important in pathogenic interactions with insects. For example, pyoverdine expression in *P. protegens* is highly upregulated once the bacterium has overcome the insect gut epithelium and is proliferating under iron-limiting conditions in the hemolymph (Vesga *et al.*, 2020).

Antimicrobial compounds

Antimicrobial compounds are a common secondary metabolite class and directly inhibit competing microbes in microbial communities. They can also provide an advantage to hosts when they target important host pathogens. For example, Actinobacteria such as *Streptomyces* are known for their production of antimicrobial secondary metabolites and are commonly found in the insect gut microbiome (Chevrette *et al.*, 2019) and in the rhizosphere (Boukaew and Prasertsan, 2014; Adegboye and Babalola, 2013; Chen *et al.*, 2018). In the genome of the plant-beneficial *S. globisporus* SP6C4 27 biosynthetic gene clusters encoding secondary metabolites were predicted (L. Pronk, unpublished results). This is in line with the finding that defence against pathogenic fungi is the main characteristic shared by the symbioses of *S. globisporus* with both strawberry plants and honeybees (Kim *et al.*, 2019). The *S. philanthi* – beewolf symbiosis is also based on antifungal compounds (Koehler *et al.*, 2013) and could explain why the symbiont was found in chili rhizospheres as a plant-beneficial species (Boukaew *et al.*, 2011). A study showing that *S. philanthi*

culture filtrates were effective in controlling rice sheath blight disease (Boukaew and Prasertsan, 2014) confirms the plant-beneficial capacities of this insect symbiont. Similarly, *P. protegens* produces toxic compounds that are important in competition with other microbes in the rhizosphere. Cyclic lipopeptides, rhizoxin and hydrogen cyanide, which contribute to *P. protegens*' biocontrol activity against soilborne diseases, were found to also play a role during insect infection (Haas and Défago, 2005; Gross and Loper, 2009). Inside the insect these toxic metabolites may provide a competitive advantage against the natural gut microflora, but also directly harm the insect host (Kupferschmied *et al.*, 2013; Loper *et al.*, 2016; Ma *et al.*, 2016; Flury *et al.*, 2017; Vesga *et al.*, 2020).

Evolutionary implications of a multi-host lifestyle

The previous sections show that some rhizosphere bacteria are capable of a multi-host lifestyle in both the rhizosphere and insects. Could this be a sign of ongoing evolution towards association with one or the other host, depending on which host provides the most benefits? Or does the adaptation to two hosts represent an evolutionarily stable lifestyle that provides more benefits than costs? As discussed above, some bacterial traits needed in rhizosphere and insect interactions are similar. Insects may frequently encounter rhizosphere bacteria and accidentally ingest them. If these bacteria are already capable of further colonizing the insect, there is a lot of potential for rhizosphere bacteria to develop more insect-specific adaptations. This makes the rhizosphere a likely source of insect symbionts, be they mutualistic, commensal, or pathogenic. In this section, we discuss examples that show how rhizosphere bacteria may evolve into specialized insect symbionts, and alternatively, how a multi-host lifestyle may be favoured by evolution.

Obligate insect symbionts may evolve from rhizosphere bacteria

The stinkbug species that were discussed in the previous sections acquire their symbiotic bacteria from the environment. In contrast, the pentatomid stinkbug species *Plautia stali* vertically transmits its symbionts from the Enterobacteriaceae family, mainly of the genus *Pantoea*, to its offspring via egg-smearing (Duron and Noël, 2016). Accordingly, most populations of *P. stali* harbour uncultivable symbionts with small genomes, a sign of coevolution (Hosokawa *et al.*, 2016). However, some populations associate with cultivable bacteria that have remarkably larger genomes and are also present in the soil habitats of their host populations (Hosokawa *et al.*, 2016). Furthermore, *P. stali* nymphs hatching from sterilized eggs

could establish a symbiosis with free-living *Pantoea* strains. Additionally, *Pantoea* symbionts of different insect species and even of different populations of the same species are only distantly related and do not form a pentatomid-specific clade (Duron and Noël, 2016; Hosokawa *et al.*, 2016). Since vertical transmission is not perfect and sometimes the original symbionts are replaced with environmental species, symbionts from different stinkbug populations and species show varying degrees of coevolution, ranging from generalist bacteria that can live outside their host to highly specialized bacteria having lost their free-living ability (Hosokawa *et al.*, 2016; Otero-Bravo and Sabree, 2021).

It would be interesting to conduct an experimental evolution study to see if plant-associated *Burkholderia* and *Pantoea* species which were able to replace the symbiotic functions of *Burkholderia insecticola* in the stinkbug *R. pedestris* (Itoh *et al.*, 2019) could evolve into more specialized insect symbionts that show signs of genome reduction similar to what is observed in other stinkbug symbionts (Takeshita and Kikuchi, 2020), and if horizontal or vertical transmission will become dominant.

A multi-host lifestyle can be a stable evolutionary state for bacteria

Does coevolution of an environmentally acquired bacterium with its host always direct towards obligate symbiosis with a single host and lead to genome erosion? Or could a multi-host lifestyle have an adaptive advantage and therefore present an evolutionarily stable alternative?

The direction of symbiont evolution along a parasite-mutualist continuum cannot be easily predicted and depends on many complex factors such as microbial community complexity, host control mechanisms and mode of transmission (see Box 1) (Drew *et al.*, 2021). A multi-host lifestyle in both plants and insects implicates a horizontal symbiont transmission mode, which is generally considered to select for parasitism, but it can also facilitate the evolution of defensive traits in mutualists (Drew *et al.*, 2021). Research on *S. philanthi*, a symbiont of beewolf wasps, shows that a horizontal transmission mode in North American beewolf populations is paired with bacterial traits that are normally associated with free-living *Streptomyces* species, such as a large and functionally diverse genome and the capability to grow in standard culture medium (Nechitaylo *et al.*, 2014). In contrast, symbionts in other, geographically separated, beewolf populations showed traits typical for strictly vertically transmitted and highly coevolved species, such as highly reduced genomes and the inability to grow in culture medium. Environmental conditions may determine the dominant mode of transmission of insect symbionts. If the insect environment is consistently nutrient-rich, and

if the symbiont is consistently transmitted to new offspring, there may be no selection pressure for the symbiont to retain its free-living capacities. This may favour an evolutionary trajectory towards high host-specificity and vertical transmission. Alternatively, if the host provides a less costly, but relatively nutrient-poor environment for its symbiont, selection may favour a high metabolic diversity that enables it to live in the environment (Nechitaylo *et al.*, 2014). This generalist lifestyle may make the switch from one host to another easier and could possibly allow for dynamic switching between multiple hosts, depending on the situation. In an often-changing environment or with an unreliable host, a multi-host lifestyle may be an adaptive trait.

In this light, acquiring a new host does not necessarily lead to a change along the parasite-mutualist continuum for the symbiont in the original host, even though there will likely be adaptations to the new host. In a recent study very closely related strains of *P. chlororaphis*, isolated from roots and from different insect species, exhibited differences in pathogenicity towards *Plutella xylostella* larvae, but not in their plant-protection abilities. A genomic analysis revealed single nucleotide polymorphisms and more complex variations (e.g. deletions, insertions) in loci related to insecticidal activity such as the Fit toxin gene cluster, a chitinase gene and a two-partner secretion system gene (Vesga *et al.*, 2021). These variations may be responsible for the differences in insecticidal activity and in the ability to colonize insects observed for the examined *P. chlororaphis* strains. An experimental evolution approach recently revealed that in the rhizosphere, *P. protegens* CHA0 can rapidly genetically adapt to a host plant. Adaptive mutations were found in genes encoding global regulators and cell surface components (LPS O-antigen) (Li *et al.*, 2021a; Li *et al.*, 2021b). Thus, one could hypothesize that *P. protegens* may also rapidly adapt to an insect host. However, *P. protegens* can live in the rhizosphere and in insects and does not show signs of a transition from a plant-associated lifestyle to an insect-associated lifestyle. When comparing *P. protegens* isolates from roots and from insects, no difference was found in their ability to colonize roots, suppress disease, and infect and kill insect larvae (Vesga *et al.*, 2021). Together with the fact that insecticidal factors such as the Fit toxin are upregulated in an insect background, while other pathways are upregulated in plants, this indicates that *P. protegens* adapted to a lifestyle in both insects and plants (Péchy-Tarr *et al.*, 2013; Vesga *et al.*, 2020). A well-equipped large genome and tight host-adapted regulatory mechanisms likely allow *P. protegens* and *P. chlororaphis* to engage in interactions with such contrasting hosts as insects and plants while limiting the energy costs for the diverse lifestyles.

For the *S. globisporus* – honeybee symbiosis, too little is known about the interaction to tell whether coevolution between the two has occurred. The interaction was ‘set up’ in an artificial environment and it is not clear whether this phenomenon also occurs in natural bee populations. Comparative genomics or transcriptomics of bee-associated and root-associated *Streptomyces* strains in both natural and experimental populations, for example, could provide more insights. Additionally, future research should point out whether the *S. globisporus* strains found on strawberry flowers and in honeybees still have plant-beneficial properties in the rhizosphere.

The evolutionary trajectory of symbiont–host interactions is not easy to predict. A multi-host lifestyle with adaptations towards both hosts appears to be a viable strategy for some plant-beneficial rhizosphere bacteria. The partly free-living lifestyle of rhizosphere bacteria in complex microbial communities may contribute to their apparent flexibility. Experimental evolution studies may provide useful insights into the mechanisms of the evolution of multi-host lifestyles and will help us understand the bigger picture of complex ecosystems.

Ecological and agricultural implications

Plant-beneficial rhizosphere bacteria and insect symbionts share many features that may allow them to switch between hosts. The ecological implications of this finding are not well understood yet and it may affect the agricultural use of rhizosphere bacteria, since the bacteria discussed above are common members of the rhizosphere microbiome of crop plants, and some are already exploited for their plant beneficial activities (Velivelli *et al.*, 2014). Thus, it is important to study not only their interactions with plants but also with insects, to decipher the ecological roles and the benefits and costs for each actor in these three-way interactions.

Insect hosts might provide nutrients to the bacteria and possibly serve as shelter, for example during overwintering. However, of particular interest is the fact that insects can transfer the bacteria from plant to plant (Nadarasah and Stavrinides, 2011; Frank *et al.*, 2017; López-Fernández *et al.*, 2017; Flury *et al.*, 2019). For plant-pathogenic bacteria it is known that they can even manipulate their host plant into attracting insect vectors (Orlovskis *et al.*, 2015), and their insect vectors into showing dispersal-related behaviour (Martini *et al.*, 2015). Similar mechanisms may be used by plant-beneficial rhizosphere bacteria, although this remains to be shown.

Plants may benefit twice if insect-vectored rhizosphere bacteria have plant-beneficial properties as well as insecticidal activity. Insect-pathogenic rhizobacteria might be dispersed by infected larvae before they die, spread from cadavers, or in the case of non-lethal infections, might be

vectored by adults emerging from colonized larvae still carrying the bacteria. Such bacteria could act as bodyguards that defend the plant against diseases and pest insects (Elliot *et al.*, 2000) and one could imagine such a function for *P. protegens*, *P. chlororaphis* or *B. thuringiensis*. Strains with this dual plant-beneficial activity could be used for biocontrol of plant diseases and pest insects. Microbe-based control of agricultural insect pests may be an environmentally friendly alternative to chemical insecticides, which are in part responsible for the worldwide decline of insect populations (Seibold *et al.*, 2019). However, the risk of accidentally infecting plant-beneficial insects (e.g. pollinators) should first be thoroughly assessed and the host specificity of the bacteria should be determined. Their level of pathogenicity may vary across insect groups, and even developmental stages. For example, CHA0 is particularly active against lepidopteran insects, and more specifically their larval stage, but is not pathogenic towards bumblebees (Péchy-Tarr *et al.*, 2008, 2013; Kupferschmid *et al.*, 2013; Ruffner, 2013; Ruffner *et al.*, 2013).

It will also be interesting to further study the ecological role of insect-mediated dispersal for plant-beneficial rhizosphere strains that show no or very weak activity against insects, such as species from the *P. fluorescens* group other than *P. protegens* and *P. chlororaphis* (Péchy-Tarr *et al.*, 2008; Flury *et al.*, 2016). Strains of the species *P. brassicacearum*, *P. thivervalensis* and *P. kilonensis*, all with potent antifungal activity, were found to colonize *Spodoptera littoralis* larvae upon oral uptake, reaching levels of up to 10^6 cfu/larva without killing the insect (Flury *et al.*, 2016). Thus, these bacteria might enter commensal interactions with the insect. If an insect vector that is only mildly damaging to a plant disperses highly effective plant-beneficial bacteria, the net effect may be positive for plant health.

From a plant perspective, plant-beneficial rhizosphere bacteria are ideally dispersed by plant-beneficial insects such as pollinators. Pollinators are already being used as 'flying doctors' to spread biocontrol products to crop plants (Biobest Group, 2021). Having pollinators spread bacteria that are both plant-beneficial and insect-beneficial would be ideal and may reduce the use of chemical pesticides and increase the health of pollinator hives while improving crop yield. For example, in the strawberry-honeybee-*Streptomyces* symbiosis (Kim *et al.*, 2019), both the plant and the insect benefit from the bacteria, which has anti-microbial properties that protect both hosts against pathogens. The dispersal of *S. globisporus* by honeybees goes from flower to flower, and it is yet unknown whether the bacteria can recolonize the rhizosphere from there (Kim *et al.*, 2019). However, honeybees that die and fall on the ground may also be a viable dispersal route. Additionally, beewolf

wasps that hunt honeybees bury their prey in the ground as food for their larvae. From there, *S. globisporus* may recolonize the rhizosphere. Interestingly, beewolf wasps also harbour *Streptomyces* symbionts, which they may acquire from the environment. It would be interesting to investigate whether *S. globisporus* can also colonize beewolf wasps, and how this affects the ecology of its other hosts.

Similar to *S. globisporus*, the *Burkholderia* symbionts of stinkbugs are both plant-beneficial and insect-beneficial. However, the insect-beneficial lifestyle of the bacterium might negatively impact the plant because stinkbugs are herbivorous. Additionally, *Burkholderia* capable of degrading the pesticide fenitrothion were found in soybean and sugarcane rhizospheres in fields that were sprayed with this chemical for pest management (Tago *et al.*, 2015). Part of the stinkbug population in these fields harboured the pesticide-degrading strains in their symbiont organ, and were therefore more resistant to the pesticide (Kikuchi *et al.*, 2012). Chemical control of stinkbugs is difficult, and symbiont-mediated pesticide resistance may enhance this problem. Given that many soil-dwelling and rhizosphere bacteria can degrade toxic compounds and are capable of horizontal gene transfer, a potential insect-associated lifestyle of such bacteria may thus have great ecological and agronomical consequences. The added possibility of insect-mediated dispersal of pesticide-degrading bacteria adds to the complexity and should be taken seriously. When introducing rhizobacteria in the field for biocontrol of plant diseases, it should be considered that those bacteria might not stay with their plant host. These bacteria potentially also associate with the insects or other fauna that are interacting with the plant, and which might disperse the bacteria into new habitats.

In this context, it is important to understand with which hosts the bacteria associate under natural conditions. The experiments with *P. protegens* CHA0 revealed that the outcome of an interaction with insects substantially depends on the insect species and can range from commensalism to pathogenicity. Despite decades of research on plant-beneficial rhizobacteria, still little is known about their specificity for certain host plants. Microbiome research revealed that different plant species and even cultivars assemble specific microbiomes (Pérez-Jaramillo *et al.*, 2016; Fitzpatrick *et al.*, 2018; Wei *et al.*, 2019). Rhizosphere bacteria with a multi-host lifestyle might have preferences for certain plant hosts and on top of that possess adaptations to certain insect host species. Future research should point out whether bacteria adapted to a certain host-plant species (e.g. cabbage) are also specifically adapted to the associated insects (e.g. herbivores feeding on cabbage). The adaptation and restriction to certain plant and insect host species might vary between

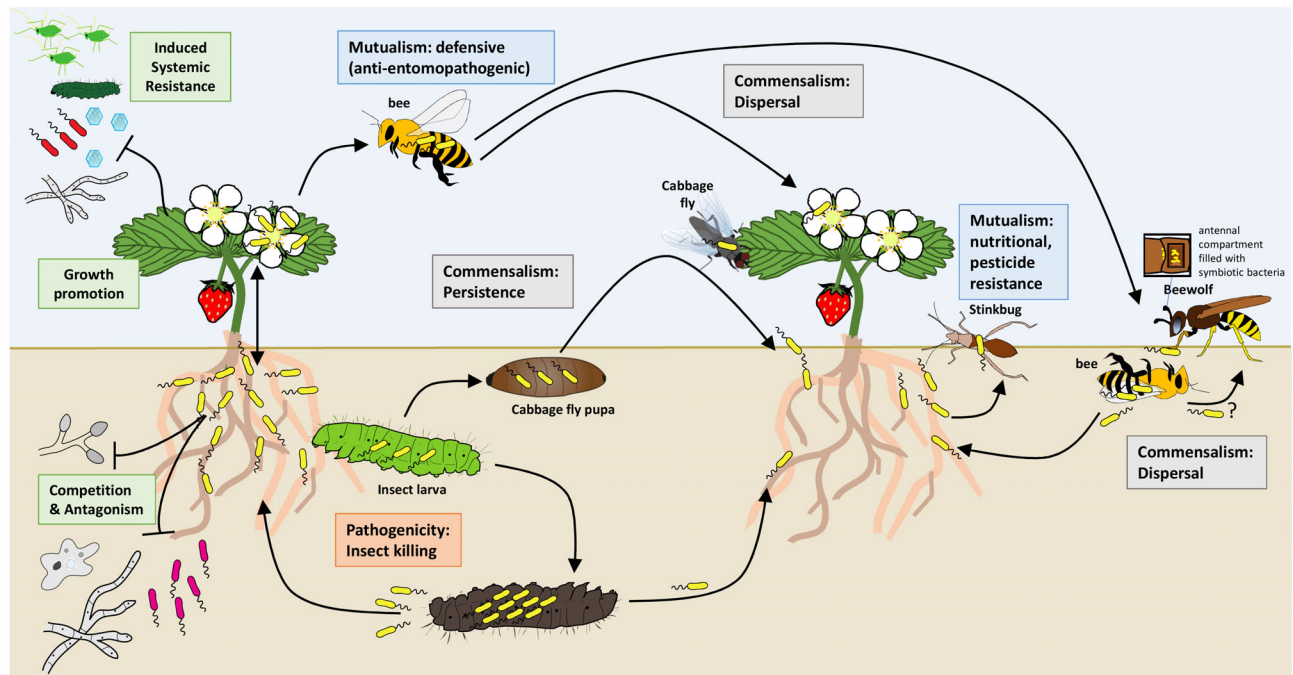


Fig. 3. Associations of rhizosphere bacteria with insect hosts might be diverse and range from mutualism to pathogenicity: Rhizosphere bacteria have multiple plant-beneficial effects. They protect the plants against pathogens by competition, antagonism and induction of plant defences. However, they can also associate with plant-interacting insects. *Pseudomonas protegens* CHA0 infects and kills lepidopteran insect larvae upon ingestion. Inside the insect body the bacteria multiply to very high numbers. From the cadaver the bacteria can recolonize plant roots. In nature, CHA0 might be rather an opportunistic pathogen, which often only persists inside insects without killing the host. In the case of the cabbage fly, CHA0 is able to persist transstadially and is dispersed by emerging adults to new host plants. Rhizosphere bacteria may move into aerial plant parts. *Streptomyces globisporus* colonizes strawberry flowers from where it is vectored by bees to new strawberry plants. It is yet unknown whether *S. globisporus* can move from the flower to the roots. Beewolves hunt honeybees and bury them in the soil. Thereby bee-associated bacteria may get access to colonize plant roots. Moreover, beewolf wasps harbour *Streptomyces pilanthi* which they acquire from the environment. The streptomycete may be capable of colonizing plant roots as well. Stinkbugs harbour *Burkholderia* symbionts that provide a nutritional benefit to the insect. The bacteria are acquired from the environment and selected by a special symbiont sorting organ in the insect.

different plant-beneficial rhizosphere bacteria as it does between different plant-pathogenic bacteria (Shikano *et al.*, 2017). Investigating these host specificities and adaptations will help to understand the complex tritrophic interactions between rhizobacteria, plants and insects. This knowledge may then be utilized to develop more sustainable and safe agricultural practices.

Concluding remarks

Recent discoveries provide experimental evidence that rhizosphere bacteria can exploit insects both as an alternative host and as dispersal vector. Additionally, there is evidence that the reported cases are not unique. Where insects and rhizosphere bacteria frequently encounter each other, interactions may be established. This is supported by the fact that many insect symbionts are closely related to rhizosphere bacteria and may deliver the same symbiotic services to their host, such as nutrient provision or disease resistance. Furthermore, whereas environmental acquisition of insect symbionts is considered rare, it does occur and may lead to relatively

stable symbioses. The genomic equipment of rhizosphere bacteria and their metabolic versatility may allow them to deal with a frequently changing environment. Even insects, which have developed mechanisms to selectively acquire their desired symbiont, can be successfully colonized by environmental bacteria. This multi-host lifestyle of rhizosphere bacteria implicates that interactions in the rhizosphere are even more complex than previously thought. In Fig. 3 we try to illustrate what these interactions may look like based on the discussed examples. Besides plant-mediated effects of rhizosphere bacteria on above-ground insects (Pineda *et al.*, 2017), direct interactions of rhizosphere bacteria with both above-ground and below-ground insects should also be considered. Future research should focus on the capacity of rhizosphere bacteria to colonize important pest insects, pollinators and other insects present in agroecosystems, and on the specificity of these interactions. Furthermore, experimental evidence of dispersal of rhizosphere bacteria by insects is so far limited to laboratory conditions with either axenic soil or only from spray-inoculated flowers to flowers. Experiments using natural soils should verify

whether bacteria established in the rhizosphere can indeed be acquired by insects and persist when they face competition of other bacteria. Another focus should lie on possible agricultural exploitation. Pathogenic rhizobacteria–insect interactions could lead to new biological pest control methods and spreading of bacteria with antifungal activity by insects might be interesting for plant protection as well.

In conclusion, plant-beneficial rhizosphere bacteria have kept their insect-associated lifestyle well-hidden for a long time. Over the last years, we have started to lift the veil covering their secrets, opening a thrilling new research field. This will lead to a greater understanding of complex ecological interactions, and it may stimulate sustainable agriculture by developing cleverer uses of bio-control bacteria.

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