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MINI REVIEW

Multiple guests in a single host: interactions across symbiotic and phytopathogenic bacteria in phloem-feeding vectors – a review

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Abstract

Some pathogenic phloem-limited bacteria are a major threat for worldwide agriculture due to the heavy economic losses caused to many high-value crops. These disease agents – phytoplasmas, spiroplasmas, liberibacters, and *Arsenophonus*-like bacteria – are transmitted from plant to plant by phloem-feeding Hemiptera vectors. The associations established among pathogens and vectors result in a complex network of interactions involving also the whole microbial community harboured by the insect host. Interactions among bacteria may be beneficial, competitive, or detrimental for the involved microorganisms, and can dramatically affect the insect vector competence and consequently the spread of diseases. Interference is observed among pathogen strains competing to invade the same vector specimen, causing selective acquisition or transmission. Insect bacterial endosymbionts are another pivotal element of interactions between vectors and phytopathogens, because of their central role in insect life cycles. Some symbionts, either obligate or facultative, were shown to have antagonistic effects on the colonization by plant pathogens, by producing antimicrobial substances, by stimulating the production of antimicrobial substances by insects, or by competing for host infection. In other cases, the mutual exclusion between symbiont and pathogen suggests a possible detrimental influence on phytopathogens displayed by symbiotic bacteria; conversely, examples of microbes enhancing pathogen load are available as well. Whether and how bacterial exchanges occurring in vectors affect the relationship between insects, plants, and phytopathogens is still unresolved, leaving room for many open questions concerning the significance of particular traits of these multitrophic interactions. Such complex interplays may have a serious impact on pathogen spread and control, potentially driving new strategies for the containment of important diseases.

Introduction

Phloem-limited bacterial phytopathogens, which are among the most devastating agricultural threats globally due to their wide host range and symptom severity, strictly rely on insect vectors to be spread from plant to plant. These pathogenic bacteria are walled Proteobacteria (α and γ subclades) and wall-less Mollicutes. The first group encompasses the α-Proteobacteria '*Candidatus* Liberibacter spp.', including important pathogens of citrus and vegetable crops (Haapalainen, 2014), and two *Arsenophonus*-related γ-Proteobacteria, namely '*Ca.* Phlomobacter fragariae' and '*Ca.* Arsenophonus phytopathogenicus' (Bressan, 2014). Plant pathogenic Mollicutes include the genera '*Ca.* Phytoplasma' and *Spiroplasma*.

All vectors of plant pathogenic bacteria residing in the phloem are Hemiptera belonging to the suborders

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Auchenorrhyncha (with the families Cixiidae, Dictyopharidae, and Flatidae in the infraorder Fulgoromorpha, and Cicadellidae in the infraorder Cicadomorpha) and Sternorrhyncha (superfamily Psylloidaea). The vectors ingest bacteria by feeding in the phloem with their piercing-sucking mouthparts. Liberibacters are transmitted by psyllids, and *Arsenophonus*-like bacteria are vectored by planthoppers in the family Cixiidae. On the other hand, phytoplasmas are transmitted by leafhoppers (family Cicadellidae), planthoppers (superfamily Fulgoroidea), and psyllids (superfamily Psylloidea), whereas spiroplasmas are vectored by leafhoppers only (Gasparich, 2010).

The interactions between plant pathogens and their vectors are not limited to a carrier-carried relation: different species or strains of a plant pathogen have divergent behaviour in different insect hosts. Moreover, phytopathogenic bacteria are included in a complex network of interactions occurring in vectors, being actual members of the multifaceted insect microbiomes, which have a significant influence on the biology of the hosts. Members of the Hemiptera, including all of the vectors of phloem-limited bacterial plant pathogens, rely on bacterial symbionts for supply of nutrients lacking in their unbalanced diet (Baumann, 2005). The nutritional provisioning operated by obligate symbionts has been a crucial condition for insect persistence and diversification on a limited food niche such as plant phloem (Skidmore & Hansen, 2017), then affecting the host range of vectors. High polyphagy deriving from mutualistic associations may in turn influence the chance of plants to be infected by a plant pathogen. Moreover, facultative symbionts are commonly found in many vectors, showing protective functions, or being capable of manipulating the host's reproduction (Zchori-Fein & Bourtzis, 2011). In addition, different species or strains of plant pathogens may be hosted by the same individual vector (Table 1), possibly being transferred together to the host plant (Bosco & D'Amelio, 2010). Such multipartite interactions most commonly result in microbial synergies or interference, with potential implications for bacterial transmission as well (Bosco & D'Amelio, 2010; Saldaña et al., 2017).

This review summarizes the knowledge concerning microbial exchanges occurring in the vectors of phloem bacterial pathogens, with special regard to the consequences on their transmission. Disease management could take advantage of these interactions to develop microbe-based control strategies (Crotti et al., 2012) (Figure 1). Indeed, despite their capability to easily adapt to, and grow in, different hosts such as plants and insects, currently these phloem-restricted bacteria cannot be cultured or are difficult to cultivate in cell-free media – with few exceptions such as spiroplasmas and a single liberibacter species (Perilla-Henao & Casteel, 2016). Such a constraint results in limited experimental exploration of new control strategies. Control is generally based on the use of healthy plant propagation material, elimination of symptomatic plants, and minimizing insect populations spreading the disease. Unravelling the interactions established between phytopathogens and insect symbionts could offer an interesting tool to impair the transmission of phloem-limited plant pathogens in a sustainable perspective.

Phloem-limited bacterial plant pathogens

Liberibacters

Transmitted by psyllids, '*Ca.* Liberibacter' pathogens include primarily obligate parasites of plants and insects, responsible for several plant diseases, among which huanglongbing (HLB) in citrus trees and zebra chip (ZC) in potatoes are the most severe ones in terms of crop damage and economic losses (Gottwald et al., 2007; Haapalainen, 2014). Three species of '*Ca.* Liberibacter' have been indicated as the causal agents of citrus HLB, previously known as citrus greening, i.e. '*Ca.* L. asiaticus' (CLas), '*Ca.* L. africanus' (CLaf), and '*Ca.* L. americanus' (CLam), the names of which were derived from the continents where they were originally found and are mainly distributed (Haapalainen, 2014). Whereas CLaf is transmitted by the African citrus psyllid, *Trioza erytreae* Del Guercio (McClean & Oberholzer, 1965), CLas and CLam are mainly vectored by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Capoor et al., 1967; Teixeira et al., 2005). *Diaphorina citri*, native to southeastern Asia, has been recently diffused in America probably in consequence of international commerce (Halbert & Núñez, 2004; Bayles et al., 2017). Despite similar symptoms after infection by each of the three HLB-causing species, CLas is the most destructive one, inducing devastating epidemics in several countries (Haapalainen, 2014). Zebra chip in potatoes and other diseases in vegetable crops are caused by '*Ca.* L. solanacearum' (CLso), which was initially named '*Ca.* L. psyllaurous' (Liefting et al., 2009). Geographically distinct CLso haplotypes are known, whose differential distribution results in the association with separate plant and insect host species. Although in North America and Oceania this pathogen is vectored by the potato/tomato psyllid, *Bactericera cockerelli* Šulc, causing severe damage in potato and tomato crops, in Europe – where it is transmitted by the psyllids *Trioza apicalis* Förster and *Bactericera trigonica* Hodkinson – it is associated with diseases of members of the Apiaceae, such as carrot and celery.

Recently, other liberibacter species have been identified, i.e., '*Ca.* L. europaeus' (CLeu) and *Liberibacter crescens* Fagen et al., but unlike the aforementioned species these latter are not reported as phytopathogens, rather showing

Table 1 Multiple bacterial infections in the vectors of phloem-limited pathogens. Only reports are listed with mixed infections in the same host individual, involving distinct plant disease agents or symbiotic bacteria with phytopathogens

Vector					
Sub- and infraorder	Family	Species	Phytopathogen multiple infection	Symbiont–phytopathogen multiple infection	Reference
Auchenorrhyncha – Fulgoromorpha	Cixiidae	*Hyalesthes obsoletus* Signoret		‘*Ca*. Sulcia muelleri’+*Wolbachia*+‘*Ca. Vidania.* fulgoroideae’+‘*Ca*. Purcelliella pentastirinorum’ +16SrXII phytoplasma	Gonella et al. (2011)
		Pentastiridius leporinus L.		‘*Ca*. Sulcia muelleri’+‘*Ca*. Purcelliella pentastirinorum’ +*Wolbachia*+‘*Ca*. Arsenophonus phytopathogenicus’	Bressan et al. (2009a)
Auchenorrhyncha - Cicadomorpha	Cicadellidae	*Amplicephalus curtulus* Linnavuori & DeLong	Phytoplasmas, groups 16SrI+16SrXII		Longone et al. (2011)
		*Amplicephalus funzaensis*Linnavuori	Phytoplasmas, groups 16SrI+16SrVII		Perilla-Henao et al. (2016)
		Circulifer tenellus (Baker)	16SrVI phytoplasma+*S. citri*		Lee et al. (1998a); Swisher et al. (2018)
		Euscelidius variegatus Kirshbaum	Phytoplasmas, groups 16SrI+16SrV	Bacterium of *E. variegatus* (BEV)+16SrI phytoplasma; *Asaia*+16SrV phytoplasma	Rashidi et al. (2014); Galetto et al. (2009); Gonella et al. (2018)
		Euscelis incisus (Kirschbaum)	Phytoplasmas, groups 16SrI+16SrIII +‘*Ca.*Phytoplasma pruni’		Orságová et al. (2011)
		Euscelis lineolatus Brulle	Phytoplasmas, groups 16SrII+16SrXII		Landi et al. (2013)
		Exitianus atratus Linnavuori	Phytoplasmas, groups 16SrI+16SrVII		Perilla-Henao et al. (2016)
		Graminella nigrifrons (Forbes)	Phytoplasmas, groups 16SrI+16SrVII, 16SrI +16SrX		Arocha-Rosete et al. (2011)
		Macrosteles sexnotatus (Fallén)		‘*Ca*. Sulcia muelleri’+*Nasuia*+16SrI phytoplasma	Ishii et al. (2013)
		Macrosteles striifrons Anufriev		‘*Ca*. Sulcia muelleri’+‘*Ca*. *Nasuia* deltocephalinicola’ +16SrI phytoplasma	Ishii et al. (2013)
		Matsumuratettix hiroglyphicus (Matsumura)		Bacterium associated with *M. hiroglyphicus* (BAMH) (*Nasuia*) +‘*Ca*. Sulcia muelleri’+16SrXI phytoplasma	Wangkeeree et al. (2012)
		Osbornellus horvathi Matsumura	‘*Ca*. Phytoplasma asteris’+‘*Ca*. Phytoplasma phoenicium’		Rizza et al. (2016)
		Paratanus exitiosus (Beamer)	Phytoplasmas, groups 16SrII+16SrVII+16SrXII		Longone et al. (2011)
		Recilia dorsalis Motschulsky		BAMH+‘*Ca*. Sulcia muelleri’+16SrXI phytoplasma	Wangkeeree et al. (2012)
		Recilia sp. nr. *vetus*		BAMH+‘*Ca*. Sulcia muelleri’+16SrXI phytoplasma	Wangkeeree et al. (2012)
		Scaphoideus titanus Ball		‘*Ca*. Cardinium hertigii’+16SrV phytoplasma	Marzorati et al. (2006)
Sternorrhyncha	Psyllidae	*Cacopsylla chinensis* (Yang & Li)	Phytoplasmas, groups 16SrII+16SrX		Liu et al. (2011)
		Cacopsylla melanoneura (Förster)		CLeu+‘*Ca*. Phytoplasma mali’	Camerota et al. (2012)
		Cacopsylla pyri L.	Phytoplasmas, groups 16SrI+16SrXII. 16SrX +16SrXII, 16SrI+16SrX	CLeu+‘*Ca*. Carsonella ruddii’+*Arsenophonus*+*Ralstonia* +‘*Ca*. Phytoplasma pyri’	Križanac et al. (2010); Raddadi et al. (2011); Camerota et al. (2012)
		Cacopsylla pyricola Förster		*Arsenophonus*+‘*Ca*. Phytoplasma pyri’	Cooper et al. (2017)
		Diaphorina citri Kuwayama		‘*Ca*. Carsonella ruddii’+‘*Ca*. Profftella aramtura’+*Wolbachia* +‘*Ca*. L. asiaticus’ (CLas); *Ralstonia*+CLas	Kruse et al. (2017); Ramsey et al. (2017); Kolora et al. (2015)
	Triozidae	*Bactericera cockerelli* (Sulc)		*Erwinia* sp.+*Wolbachia*+*Staphylococcus* sp. +*Enterococcus* sp.+CLso	Kolora et al. (2015)
		Bactericera trigonica Hodkinson	‘*Ca.*Liberibacter solanacearum’ (CLso) +phytoplasmas, groups 16SrVI+16SrI		Swisher et al. (2018)

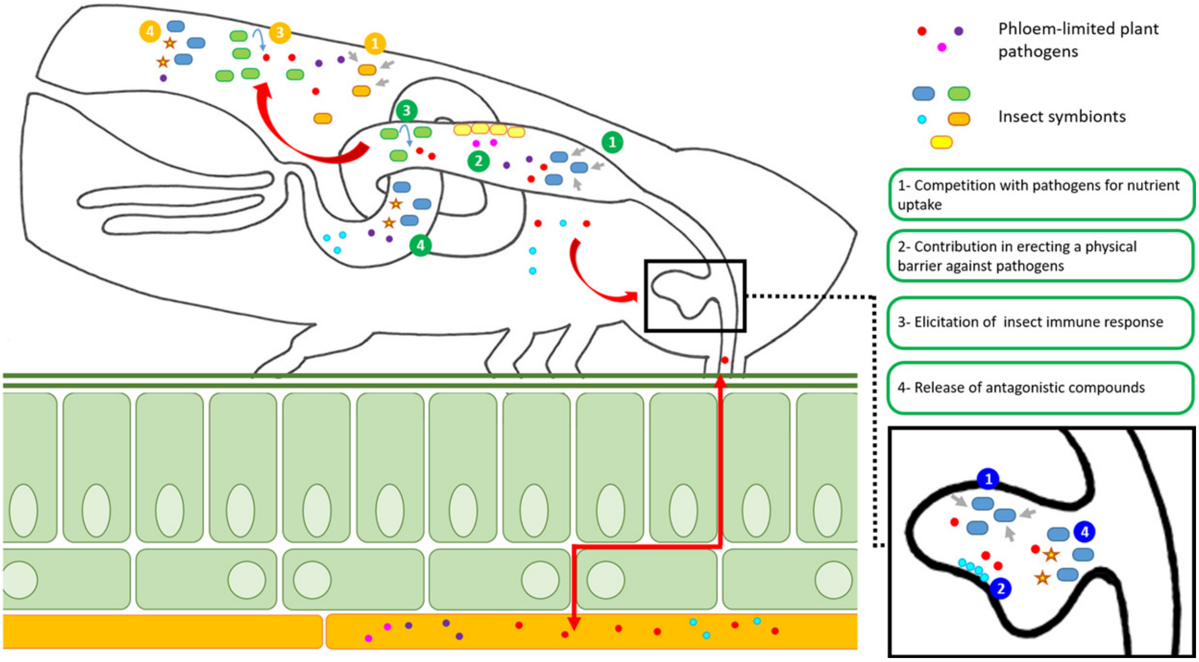

Figure 1 Insect symbionts could be useful for controlling the transmission of phloem-limited plant pathogens. Phloem-restricted plant pathogens are indicated as red, purple, or violet dots, whereas microbial symbionts are depicted with other different colours and shapes. Microbe movement is indicated with red arrows. Symbiont-mediated control mechanisms (referred as control systems) of pathogen transmission are listed on the right and corresponding numbers are depicted in the gut (in green dots), hemolymph (in orange dots), and salivary glands (inset, in blue dots). Grey arrows indicate competitive nutrient uptake by symbiotic bacteria (control system 1), blue arrows depict symbiont-mediated immune response of the insect (control system 3), and stars represent released antagonistic compounds (control system 4).

an endophytic behaviour (Raddadi et al., 2011; Leonard et al., 2012). Interestingly, *L. crescens*, found in mountain papaya in Puerto Rico, can be grown in axenic cultures, making it an ideal candidate to study liberibacters' biology (Leonard et al., 2012; Fagen et al., 2014a,b). CLeu, reported as an endophyte of pear, apple, blackthorn, and hawthorn, transmitted by *Cacopsylla* spp. (Raddadi et al., 2011; Camerota et al., 2012), was recently indicated as a pathogen in Scotch broom, *Cytisus scoparius* (L.) Link, in New Zealand (Thompson et al., 2013). Two other new candidate liberibacter species were recently reported: '*Ca.* Liberibacter caribbeanus' (CLca) detected in *Citrus sinensis* (L.) Osbeck and in the citrus psyllid, *D. citri*, from Colombia (Keremane et al., 2015) and '*Ca.* Liberibacter brunswickensis' (CLbr) detected in the native Australian eggplant psyllid, *Acizzia solanicola* Kent & Taylor (Morris et al., 2017). Neither of these new species was related with plant disease but an association with psyllids as secondary symbionts is inferred (Morris et al., 2017).

***Arsenophonus*-like bacteria**

The genus *Arsenophonus* includes not only plant pathogens, but also insect parasites and symbionts (Bressan, 2014). For instance, in a survey performed on 136 arthropod species, *Arsenophonus* bacteria were found to be associated with 5% of the tested hosts (Duron et al., 2008), where they can establish complex interactions with beneficial or parasitic features (Wilkes et al., 2011). Conversely, two species cause disease to strawberry and sugar beet plants (Danet et al., 2003; Bressan et al., 2008). The first pathogenic agent was discovered at the end of last century in France on strawberries affected by marginal chlorosis. Because at that time very little was known about this genus, the pathogen was considered as a separate species that was named '*Ca.* Phlomobacter fragariae' (Zreik et al., 1998), but based on sequence data it was subsequently proposed to be an *Arsenophonus* (Bressan, 2014). The other plant pathogenic *Arsenophonus* is '*Ca.* Arsenophonus phytopathogenicus' which infects sugar beet, causing a disease defined as 'basses richesses' syndrome, because diseased plants show decreased sugar content (Richard-Molard et al., 1995). The insect vectors of pathogens in the *Arsenophonus* group are cixiids: '*Ca.* Phlomobacter fragariae' is vectored by *Cixius wagneri* (China) (Danet et al., 2003), whereas '*Ca.* Arsenophonus phytopathogenicus' is transmitted by *Pentastiridius leporinus* (L.) (Gatineau et al., 2002). These two pathogens are phylogenetically distinct, and can differentially interact with plants and insects in different contexts. In Italy '*Ca*. Arsenophonus phytopathogenicus' was observed to be related to a strawberry marginal chlorosis disease (Terlizzi et al., 2007); likewise it was detected in *C. wagneri*, which was able to inoculate it to sugar beet plants, whereas strawberries were not infected (Bressan et al., 2008). The epidemiology of this group of diseases is complicated by the fact that they can be induced also by phytoplasmas transmitted by *Hyalesthes obsoletus* Signoret (Gatineau et al., 2002; Danet et al., 2003). Even being plant pathogens, there is evidence that many traits of *Arsenophonus*-like bacteria are characteristic of an insect symbiont lifestyle, such as reproductive tissue colonization and vertical transmission, absence of entomopathogenic activity, high infection rate, and a life cycle prevalently related to insect hosts (Bressan et al., 2009b; Bressan, 2014). Thus, these bacteria could easily initiate new associations with additional cixiid species. The complexity of their associations with insects and plants, together with cixiids' capability to easily adapt to new environments and host plants, could effectively explain the increasing appearance of emerging *Arsenophonus*-related diseases.

Phytoplasmas

Phytoplasmas are known to be responsible for diseases in over 1 000 economically important crops worldwide (Marcone, 2014). Typical symptoms include yellowing, witches' broom, virescence, phyllody, bolting, reddening of leaves and stems, decline, and stunting of plants (Hogenhout et al., 2008). To date, all known phytoplasmas are reported to be pathogenic for at least one plant, even though asymptomatic hosts may be recruited. Phytoplasma taxonomy has been hampered by their recalcitrance to be cultured in vitro, therefore these bacteria are partially classified in the provisional genus '*Ca.* Phytoplasma' based on sequence analysis. Up to now 42 '*Ca.* Phytoplasma' species have been reported (Zhao & Davis, 2016). A more exhaustive categorization defines phylogenetic clusters (16SrI-XXXIII groups, each divided in many subgroups) based on 16S *rRNA* gene sequences (Lee et al., 1993, 1998b; Zhao & Davis, 2016).

As most phytoplasmas cause symptoms to plants belonging to different families, such phytopathogens are regarded as some of the most troubling disease agents in affected areas. Some phytoplasmas are successfully transmitted by polyphagous vectors, further incrementing their chance to infect a huge number of plants. For example, Aster Yellows phytoplasmas (16SrI) are vectored by many polyphagous leafhoppers to several plants (Weintraub & Beanland, 2006), including flowers, vegetables, or grapevine. The broad range of wild and cultivated plants that are affected by these pathogens can be explained by the polyphagy recorded for most of the vectors, along with the great diversity of phytoplasma subclades (Hogenhout et al., 2008).

Considering vector-phytoplasma interplays, many specific interactions are acknowledged between different phytoplasma phylogenetic groups and distinct taxa of vectors. As an example, only cicadellid leafhoppers have been reported to transmit phytoplasmas of the 16SrI group (Alma et al., 2015). On the other hand, many phytoplasmas are indistinctively vectored by distant insects. For instance, phytoplasmas of the phylogenetic groups 16SrV and 16SrXII may be vectored by Fulgoromorpha and Cicadomorpha, and 16SX phytoplasma by Auchenorrhyncha and Sternorrhyncha (Alma et al., 2015). However, a single family with major vector importance can be generally recognized even for pathogens transmitted by distinct taxa: most vectors of 16SrV phytoplasmas belong to the Cicadellidae, 16SrXII phytoplasmas are mainly transmitted by cixiids, and 16SrX phytoplasmas by psyllids.

Spiroplasmas

Spiroplasmas are an extremely harmful group for global agriculture, even though only few species have been accounted as phytopathogens, i.e., *Spiroplasma citri* Saglio in citrus, *Spiroplasma kunkelii* Whitcomb et al. in maize, and *Spiroplasma phoeniceum* Saillard et al. in aster (Gasparich, 2010). Insects are major reservoirs for spiroplasmas, some of which are strictly insect symbionts (Gasparich, 2010). All plant pathogenic spiroplasmas are phylogenetically related, being included in the Citri clade (Gasparich, 2010). Although spiroplasmas and phytoplasmas establish similar pathogenic relationships with host plants, inducing analogous symptoms, major biological differences are evident between these genera. Distinctions include their shape – spiroplasmas are helical, phytoplasmas are pleomorphic – and cultivation suitability – spiroplasmas can be cultured in nutrient-rich media, phytoplasmas are recalcitrant to cultivation (Gasparich, 2010).

Spiroplasma citri is mainly related to heavy losses in citrus production; however, this pathogen, as well as its vectors, may be found on many different host plants. *Spiroplasma citri* is the agent of citrus stubborn, brittle root disease of horseradish, sesame yellowing, and carrot purple leaf (Zarei et al., 2017). It is transmitted by the leafhoppers *Circulifer haematoceps* (Mulsant & Rey) in the Mediterranean basin and *Circulifer tenellus* (Baker) in North America (Renaudin, 2006). The main areas affected by *S. citri*-related diseases are the Mediterranean countries of Europe, North Africa, and western Asia, as well as the Nearctic region, whereas the pathogen is absent in South America.

Spiroplasma kunkelii is restricted to the Americas, where it is an important pathogen of maize crops. Its natural vector is the cicadellid *Dalbulus maidis* (Delong & Wolcott), a specialist of the genus *Zea* in the Nearctic and Neotropical areas. *Dalbulus maidis* underwent strict co-evolution with maize, being among the most prevalent leafhoppers in this crop (Palomera et al., 2012).

Spiroplasma phoenicium was retrieved from periwinkle plants affected by yellows in Syria. This pathogen is experimentally transmitted by the leafhopper *Macrosteles fascifrons* (Stål); however, at present no information is available concerning the natural vectors of *S. phoeniceum* in the infested area (Saillard et al., 1987).

Bacterial phytopathogen-vector relations

Vectors transmit the phloem-restricted pathogens in a persistent manner: once ingested by feeding on infected plants, bacterial cells multiply in the insect midgut, cross the epithelium, replicate in the hemolymph, and ultimately infect the salivary glands so that they are injected in the new host plant (Figure 1; Gasparich, 2010; Bressan, 2014; Haapalainen, 2014). This process implies complex interplays, spanning from beneficial to adverse. A benign role was suggested for CLas in *D. citri* (Duan et al., 2009; Mann et al., 2011), although infected psyllids were found to be more susceptible to selected insecticides, resulting in fitness decrement in given circumstances (Mann et al., 2011). Similarly, a negative density-dependent effect of CLso infection on the fecundity of *B. cockerelli* was reported by Nachappa et al. (2014), whereas no significant detrimental effects on the biology of infected individuals occurs according to Thinakaran et al. (2015). Effects of vector manipulation by a phytopathogen have been observed also at the hemolymph level, as in CLas-infected *D. citri* showing changes in proteins related to energy metabolism, immunity, and lipid transport (Kruse et al., 2018). Differential effects have been reported for insect-phytoplasma associations: for example, shorter survival and lower egg production were observed in individuals of *Scaphoideus titanus* Ball infected by 16SrV phytoplasmas (Bressan et al., 2005), whereas a positive influence was recorded for 16SrI phytoplasmas in *Macrosteles quadrilineatus* DeLong & Caldwell (Beanland et al., 2000).

The molecular mechanisms regulating plant pathogen retention, multiplication, and spread in some vector species, and not in others, are still poorly understood. Adaptation of vectors to harbour plant pathogens suggests co-evolution between insects and bacteria; however, insect–bacterium interactions have polyphyletic traits, indicating multiple independent evolutionary events (Orlovskis et al., 2015). The evolution of pathogen transmission shares some traits with insect symbiosis, as most plant pathogens are phylogenetically related to many symbiotic bacteria of Hemiptera and, like endosymbionts, they have reduced genomes, reflecting the adaptation to obligate associations (Bendix & Lewis, 2018). Indeed, a major consequence of a host-dependant life style is extreme gene loss, due to the lack of a selection process to maintain genes that are superfluous in the rich environment provided by the insect body (Latorre & Manzano-Maríın, 2017). In most cases, the associations between plant pathogens and their vectors are thought to originate from bacterial internalization and successful survival in insects feeding transiently in infected plants (plant-first model). Conversely, some phytopathogens, especially those in the Enterobacteriaceae, may have been initially insect commensals (i.e., non-harmful associates) that have evolved as plant pathogens following repeated inoculations in the phloem by their insect hosts (insect-first model) (Bové & Garnier, 2002; Nadarasah & Stavrinides, 2011).

Traits affecting vector suitability and specificity are thought to be related to differences in insect physiology, immunity, and behaviour, as well as to their geographical and seasonal distribution (Perilla-Henao & Casteel, 2016). For instance, divergent plant host-dependant feeding behaviour has been suggested to play an important role in differential transmission competence in the leafhopper phytoplasma vectors *Euscelidius variegatus* (Kirschbaum) and *Empoasca decipiens* Paoli (Galetto et al., 2011). The vector immune system may limit pathogen invasion: CLas acquisition by adult *D. citri* was proven to be less efficient than by nymphs due to differential immune responses, like melanization and apoptosis of gut cells (Kruse et al., 2017). Similarly, immune response may be the cause of limited phytoplasma cell numbers found in non-transmitting individuals of various vector species after experimental exposure to the pathogens (Galetto et al., 2009). A crucial phase of the transmission process is the protein interaction between pathogen cells and those of the host, regulating pathogen crossing of gut and salivary gland epithelia. The main strategy for bacterial internalization reported for plant pathogenic agents is endo-exocytosis (Kwon et al., 1999; Hogenhout et al., 2008; Cicero et al., 2016), mediated by various membrane proteins (Labroussaa et al., 2010, 2011; Béven et al., 2012; Duret et al., 2014; Konnerth et al., 2016; Arricau-Bouvery et al., 2018). The absence of specific adhesion machinery to host cells seriously weakens the vector competence (Weintraub & Beanland, 2006). For example, *S. citri* strains lacking adhesion-related proteins are not transmissible by insects (Kruse et al., 2017).

The transmission of a plant pathogen by vectors is affected also by the fact that different species or strains of the pathogen differ behaviourally in different insect hosts. This is especially observed for those phytopathogens that most probably derive from insect symbionts, such as *Arsenophonus* bacteria and spiroplasmas. Both the genera *Arsenophonus* and *Spiroplasma* encompass inter- and intracellular symbiotic bacteria displaying a diversity of roles, from mutualism to reproductive manipulation, or they may even be entomopathogenic (Gasparich, 2010; Bressan, 2014). In '*Ca*. A. phytopathogenicus' and '*Ca*. P. fragariae', it has been shown that the exploitation of plants resulted from independent evolutionary events from a common endosymbiotic ancestor (Bressan, 2014). This evidence, along with the observation of typical symbiotic traits in insects, like high prevalence and maternal transmission, suggests the transition from endosymbiotic to plant pathogenic life style (Bressan, 2014). Species belonging to other phytopathogen groups could actually derive from insect commensals. For example, phylogenetic studies demonstrated a match between the affinity level of liberibacter species restricted to different continents and the geographical distribution of psyllid hosts. This supported the hypothesis of co-evolution between CLbr, behaving as an insect secondary symbiont, and its host *A. solanicola* (Morris et al., 2017). On the other hand, co-evolved associations of a plant pathogen and an insect vector may lead to mitigate harmful effects on host fitness (Purcell, 1982). The growing number of observed transitions from insect endosymbiosis to pathogenesis in plants and vice versa is certainly indicative of the possibility that new bacterial species, currently thought to be horizontally transmitted insect commensals or mutualists, will become emerging plant pathogens in the future.

From a disease containment perspective, the enhancement of insect immunity could be a specific control objective in case of phytopathogen-vector interactions where the bacterium is recognized and attacked by insect immune cells (Weiss & Aksoy, 2011). In contrast, some phytopathogens are able to escape the immune response. For example, *S. citri* has been reported to evade phagocytosis and limit phenoloxidase activity in its vector *C. haematoceps* (Eliautout et al., 2016). In those cases, control approaches based on immune augmentation may be insufficient.

Multiple pathogen infections and competition

The interaction among pathogens, plants, and vectors can be complex. Mixed infections by bacterial pathogens are commonly observed in the phloem of a single plant. The simultaneous occurrence of multiple pathogens, either related or phylogenetically distant, is rather frequent in single herbaceous plants and trees of many families (Križanac et al., 2010; Nicolaisen et al., 2011; Arratia-Castro et al., 2016; Satta et al., 2016; Swisher et al., 2018). Throughout its life cycle, a single insect may feed on several plants of the same or different species, probably being exposed to mixed pathogen infections. As a consequence, insect vectors may acquire many pathogen species or strains during a feeding event, or by feeding sequentially on host plants infected by different bacteria (Križanac et al., 2010; Raddadi et al., 2011; Swisher et al., 2018) (Table 1). In some cases, the co-occurrence of multiple pathogens in an insect's body is inhibited by interferential interactions such as selective acquisition or transmission of a single microbe (Bosco & D'Amelio, 2010). For example, in the leafhopper *D. maidis*, the natural vector of maize bushy stunt phytoplasma (MBSP) and corn stunt spiroplasma (CSS), competition for transmission was reported after co-occurrence during a long-term latency period (de Oliveira et al., 2007). This competition resulted in suppression of prolonged transmission of MBSP after acquisition of CSS, as the latter is thought to have higher rates of multiplication and spread, hence being more competitive during the latency period required for successful transmission. Similar results were obtained with the cicadellid *M. quadrilineatus*, vector of several strains of Aster Yellows Phytoplasma. Leafhoppers exposed to sequential acquisition of different phytoplasma strains most frequently transmitted the first provided isolate exclusively (Freitag, 1976). This evidence suggests competitive colonization of the insect's body, where the first strain starting multiplication and reaching the salivary glands is more competitive and hence transmitted preferentially (Bosco & D'Amelio, 2010). The same competitive colonization process was proposed for *Osbornellus horvathi* Matsumura, as adult leafhoppers double-infected with '*Ca*. P. asteris' and '*Ca*. P. phoenicium' were able to transmit the former, but not the latter, to various plants under experimental conditions (Rizza et al., 2016). Considering *Arsenophonus*-related plant pathogens, no specific transmission trial from double-infected sources has been reported yet; however, there is evidence that separated populations of *C. wagneri*, the only known vector of both pathogens, exclusively transmit '*Ca*. A. phytopathogenicus' or '*Ca*. P. fragariae', but do not carry the two bacteria together (Bressan et al., 2008). Many factors must be taken into account to explain exclusive pathogen acquisition by *C. wagneri*, including vector ecology and population dynamics, which could lead to limited chance for the same individual to be exposed to both pathogens; however, the competition between '*Ca*. A. phytopathogenicus' and '*Ca*. P. fragariae' for insect colonization cannot be ruled out.

Competition between two bacterial pathogens in a vector has been dissected by Rashidi et al. (2014), using the leafhopper *E. variegatus* and two unrelated phytoplasmas, chrysantheum yellows phytoplasma (CYP) and flavescence dorée phytoplasma (FDP), experimentally transmitted to broad bean plants. Insects sequentially exposed to CYP and FDP displayed unilateral interference, with the suppression of FDP transmission regardless of the feeding order. Acquisition of a pathogen was not affected by the presence of the other one, suggesting no competition at the earlier infection stages. Competition was rather identified to take place in the salivary glands, which were more rapidly invaded by CYP as it multiplied faster than FDP, even though the latter bloomed to higher concentrations. The higher speed in reaching salivary glands displayed by CYP was suggested to be related to its (1) long co-evolutionary history with the insect host and the consequently mitigated immune response, and (2) broad phytoplasma host range supporting the evolution of traits that promote acceptability by a broad vector range (Rashidi et al., 2014). Transcriptomic analysis of infected leafhoppers with single phytoplasma strains demonstrated the stimulation of insect immune response (by activation of the Kazal type 1 serine protease inhibitor and melanization pathway) after infection by FDP, which reduces host fitness and is then perceived as a potential pathogen (Galetto et al., 2018). Instead, the most competitive CYP increased energy metabolism, suggesting that the host tolerates fast multiplication rates in response to the mutualistic behaviour exhibited by this strain.

Understanding the competition between co-occurring pathogen strains in the same host, although scarcely studied, could support the study of pathogen transmission. Observation and characterization of competition events may contribute to unravel the processes determining insect invasion and spread of phytopathogens, possibly identifying weaknesses of single associations and revealing new control targets. Moreover, competitive transmission of plant pathogens may seriously alter disease epidemiology in the field.

Symbiont–pathogen interactions

The groups of Auchenorrhyncha (leafhoppers, planthoppers, froghoppers, and treehoppers) and Sternorrhyncha (aphids, whiteflies, psyllids, mealy bugs, and scale insects), include all of the vectors of plant pathogenic bacteria. These insects harbour both obligate and facultative endosymbionts which play important roles in supplying nutrients and providing the host with other fitness benefits (Baumann, 2005; Morrow et al., 2017). The main obligate (primary) symbionts are '*Ca*. Sulcia muelleri' in Auchenorrhyncha, and '*Ca*. Carsonella ruddii' in psyllids. Moreover, *Sulcia* requires complementary (co-primary) symbiotic bacteria to integrate its nutrient supply to the insect (McCutcheon & Moran, 2010). Similarly, psyllids harbour secondary symbionts, such as *Sodalis* or *Arsenophonus* bacteria, with nutritional roles (Morrow et al., 2017). The function of some symbionts of hemipteran vectors is still unrecognized. For example, many bacteria generally known as reproductive manipulators, such as *Wolbachia*, *Cardinium*, *Rickettsia*, and *Arsenophonus*, have been found in several vector species; however, their role has not been characterized yet (Marzorati et al., 2006; Gonella et al., 2011; Jing et al., 2014; Iasur-Kruh et al., 2017; Morrow et al., 2017). Some insect beneficial microorganisms (e.g., *Rickettsia* and *Cardinium*), capable of colonizing the salivary glands, may be transferred from insect to plant and vice versa, possibly establishing endophytic relationships as well (Caspi-Fluger & Zchori-Fein, 2010; Gonella et al., 2015; Iasur-Kruh et al., 2017). Despite the recognition that microbial communities affiliated to non-model insects need to be studied (Prosdocimi et al., 2015), which recently led to growing evidence of co-existence of plant pathogens and other microbes in insect vectors, few studies directly investigated their interactions (Table 2). Symbiont-pathogen exchanges were first studied in psyllids, specifically in the CLas vector *D. citri*. This psyllid harbours three main endosymbionts: a species of *Wolbachia*, the γ-Proteobacterium '*Ca*. Carsonella ruddii' – an endosymbiont which may provide nutritional benefits to its host (Thao et al., 2000) –, and '*Ca*. Profftella armatura' – a β-Proteobacterium with defensive function (Nakabachi et al., 2013). Fagen et al. (2012) first observed a negative correlation between CLas infection rate and the relative abundance of *Profftella* within the microbial community. Based on its genome sequence, *Profftella* was predicted to produce defensive toxins, i.e., diaphorin and diaphorin-related polyketides. CLas-infected [CLas(+)] insects were found to have dramatically elevated levels of two proteins involved in polyketide biosynthesis. In contrast, the protein responsible for initiating diaphorin biosynthesis is down-regulated in CLas(+) *D. citri* (Ramsey et al., 2015). Moreover, Ramsey et al. (2015) observed that the ratio between levels of diaphorin and the related polyketide is significantly increased in CLas(+) compared to CLas uninfected [CLas (−)] *D. citri*, suggesting changes in *Profftella* polyketide metabolism in response to the presence of the pathogen or in direct or indirect response to changes induced by the pathogen in infected plants. The up-regulation of the *polyketide synthase* (*PKS*) gene expression in CLas(+) *D. citri* may be a specific response of *Profftella* to the presence of CLas, as part of an infection response that may be

Table 2 Symbiont–pathogen interactions reported in the vectors of phloem-limited plant pathogenic bacteria

Insect	Phytopathogen	Symbiont	Interaction	Reference
Diaphorina citri	'*Ca.* Liberibacter asiaticus' (CLas)	'*Ca.* Profftella armatura'	Upregulation of genes involved in biosynthesis of diaphorin polyketide	Ramsey et al. (2015)
		Wolbachia	Positive correlation	Fagen et al. (2012)
Hyalesthes obsoletus	16SrXII phytoplasma	*Dyella*-like bacterium (DLB)	Reduction of phytoplasma-related symptoms in grapevine	Iasur-Kruh et al. (2018)
Euscelidius variegatus	16SrV phytoplasma	*Asaia* sp.	Reduced phytoplasma acquisition in *Asaia*-infected individuals	Gonella et al. (2018)
Matsumuratettix hiroglyphicus, *Recilia dorsalis*, *Recilia* sp. nr. vetus	Phytoplasmas	Bacterium associated with *M. hiroglyphicus* (BAMH) (*Nasuia*)	BAHM suggested to be required for successful phytoplasma transmission	Wangkeeree et al. (2012)
Dyctiophara europaea	16SrV phytoplasma	*Wolbachia*	Mutual exclusion	Krstić et al. (2018)

mediated by *D. citri* (Ramsey et al., 2015). Such an interactive response may involve *Carsonella* as well, which could provide the host with essential amino acids required for polyketide production (Ramsey et al., 2015).

Besides psyllid-liberibacter interactions, further evidence of antagonistic relationships between symbiotic bacteria and plant pathogens is reported for some Auchenorrhyncha vectors of phytoplasmas. A bacterium in the Xanthomonadaceae, provisionally named *Dyella*-like bacterium (DLB) (Iasur-Kruh et al., 2017), was isolated from the planthopper *H. obsoletus*, and showed anti-phytoplasmal activity in inoculated plants (Iasur-Kruh et al., 2018). Despite being isolated from an insect source, DLB showed endophytic traits: it was consistently found in the wild bush *Vitex agnus-castus* L., and it was able to long-term colonize the phloem of various plant species, including many hosts of phytoplasmas and liberibacters (Lidor et al., 2018). Once established in grapevines infected by phytoplasmas, DLB reduced disease symptoms (Iasur-Kruh et al., 2018). Based on DLB genome analysis, the authors suggested that such a drop of symptoms is related to inhibition of pathogens, rather than competition or production of substances stimulating plant growth or defence (Lahav et al., 2016; Iasur-Kruh et al., 2018). Moreover, DLB was demonstrated to inhibit the growth of the cultivable model Mollicutes *Spiroplasma melliferum* Clark et al. (Iasur-Kruh et al., 2017).

Acetic acid bacteria in the genus *Asaia* are widespread in insects, including leafhoppers transmitting phytoplasmas, and they were proposed to interact with insect vectors, possibly altering their spread (Crotti et al., 2009). Strains with different phenotypes previously isolated from mosquitoes were orally supplied to the experimental vector of FDP, *E. variegatus*, which was successfully colonized. One *Asaia* strain producing an air-liquid interface biofilm, after establishing in *E. variegatus*, reduced its acquisition of FDP from broad beans under experimental conditions (Gonella et al., 2018). These authors suggested that the strain of *Asaia* could affect the capability of the phytoplasma of crossing the gut epithelia in order to reach the salivary glands, even though the mechanisms regulating this interference remain to be elucidated. However, such an alteration was imperfect and, when the pathogen succeeded in colonizing the insect, transmission rates to broad beans were similar to those recorded for control leafhoppers unexposed to *Asaia* (Gonella et al., 2018).

Additional interplays between symbiotic bacteria and plant pathogens have been suggested by multiple prevalence studies, as in some cases positive correlation or mutual exclusion could be detected between symbiotic and phytopathogenic bacteria. For example, the obligate symbiont *Nasuia*, widespread in the family Cicadellidae, is present in most leafhopper species transmitting phytoplasmas, whereas non-vector species were shown to lack it (Wangkeeree et al., 2012). It has been suggested that *Nasuia* could be required for successful transmission. Likewise, in the planthopper FDP vector, *Dictyophara europaea* L., a negative correlation between infections by phytoplasma and *Wolbachia* was reported, suggesting that the *Wolbachia* strain infecting *D. europaea* displays antagonistic activities against the pathogen, or alternatively competes for insect colonization (Krstić et al., 2018). In *D. citri* an increase in the ubiquitous *Wolbachia* titre was reported with CLas infection (Fagen et al., 2012), indicating a more complicated interplay mechanism with strain-specific variability. Direct interaction has been documented between *Wolbachia* and CLas, as the first suppresses the holing lytic promoter in a CLas-infecting phage in *D. citri* (Jain et al., 2017).

The studies of synergies and interferences between symbiotic agents and plant pathogens offer significant cues for disease treatment; still, further work is required to describe new interactive associations. Future work concerning such interplays should be aimed not only at identifying direct anti-pathogen activity expressed by symbionts, but also at altering the mutualistic exchange recorded among vectors, symbionts, and phytopathogens, and to influence insect ecology (e.g., by driving plant choice and governing interactions with stresses).

Conclusions and open issues

The interactive roles of phytopathogenic and symbiotic bacteria in insects represent an emerging topic. The bacterial interactions occurring in insects also affect the life cycle of the host. Considering the reported transition of disease agents in their vectors from symbiont to phytopathogen, the effects of these bacteria are key for the study of insect–microbe relationships; however, they are still mostly unknown. Such effects may result in uneven competitive behaviour described for both closely and distantly related pathogens. Various questions arise from this hypothesis. How is insect immunity involved in differential growth rates of plant pathogens? What traits of vector–pathogen interactions originate diversity in host responses? Are these bacteria at different steps of transition from symbiont to pathogen or vice versa (e.g., do the most competitive pathogens supply the host with fitness advantages)? Galetto et al. (2018) addressed several of these questions using the *E. variegatus*-CYP-FDP model, but the analysis of competitiveness conditions needs to be expanded to other pathogens and vectors. Moreover, it is still unclear whether non-competitive or beneficial interactions take place among pathogens in insects with multiple infections. And how are plants implicated in these interactions? Many examples exist of the effects of phytopathogens on plant processes in favour of insects, such as the promotion of insect attraction to infected hosts, stimulating the spread of the pathogens (Orlovskis et al., 2015). However, whether pathogens that are capable of modulating their attractiveness are stronger competitors than horizontally transmitted microbes (including other phytopathogens) is poorly understood. The elucidation of molecular and cellular machineries of insect-phytopathogen-host plant relations could help answer these issues.

Additional open questions involve the role of insect bacterial endosymbionts in plant pathogen competition and spread. Only few examples of interactions between symbionts and pathogens have been described, in spite of the high number of symbiotic bacteria depicted in most vectors: direct evidence of interference with the transmission process in the insect or with symptom development in the plant have been provided only for phytoplasmas (Gonella et al., 2018; Iasur-Kruh et al., 2018). The mechanisms regulating beneficial or hostile exchanges have been only rarely elucidated, and some bacterial pathogens were shown to exhibit mutualistic effects on their vectors, whereas others caused fitness costs (Hogenhout et al., 2008; Tamborindeguy et al., 2017). An open field for future research is whether harmful or beneficial roles are in some way the result of interactions with bacterial symbionts co-inhabiting the same host. An indirect effect on the insect fitness as a consequence of symbiont suppression was observed in virus-transmitting aphids. In the soybean aphid, *Aphis glycines* Matsumura, a drop in the concentration of endosymbiotic *Buchnera* was observed after exposure to Bean pod mottle virus, resulting in reduced aphid fecundity (Cassone et al., 2015).

A still unexplored field of research is the manipulation of insect endosymbiotic microbes in vectors by means of paratransgenesis, to drive their interaction with phloem-limited plant pathogens towards antagonistic activities. A similar approach was proposed, for example, for *Xylella fastidiosa* Wells et al., a xylem-restricted pathogen causing Pierce's disease to grapevine; *Alcaligenes xylodoxidans* subsp. *denitrificans* (Rüger & Tan) Kiredjian et al., a bacterium reported as an insect symbiont and an endophyte, was proposed as a candidate for genetic transformation to display anti-*Xylella* molecules (Bextine et al., 2004).

Along with being of interest to elucidate biological mechanisms regulating insect–bacteria relationships, microbial interactions occurring in insect vectors have important implications for disease epidemiology and control. From the epidemiological point of view, competition among plant pathogens alters the rates of transmission by vectors, and possibly influences their fitness as well, with an impact of the spread of diseases on multiple plants. From the angle of disease control, the study of microbial interactions in vectors could provide valuable tools to manage crop infections by altering vector competence via symbiotic control approaches (Alma et al., 2010). Possible strategies include the identification of detrimental effects by symbionts on plant pathogens in the insect, or the selection of new molecular targets to interrupt beneficial interplays among bacteria.

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