www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 2752-2762

www.thepharmajournal.com
Received: 24-04-2022
Accepted: 29-05-2022

Shahid Mushtaq
SKUAST Kashmir,
Jammu and Kashmir, India

Baby Summuna
SKUAST Kashmir,
Jammu and Kashmir, India

FA Bhat
SKUAST Kashmir,
Jammu and Kashmir, India

Vikas Gupta
SKUAST Kashmir,
Jammu and Kashmir, India

Addafar Gulzar
SKUAST Kashmir,
Jammu and Kashmir, India

Aabid Hussain Sheikh
IARI, New Delhi,
Delhi, India

Syed Muzamil
SKUAST Kashmir,
Jammu and Kashmir, India

Corresponding Author
Shahid Mushtaq
SKUAST Kashmir,
Jammu and Kashmir, India

Vector transmitted bacterial diseases and their interaction with hemipteran insects

Shahid Mushtaq, Baby Summuna, FA Bhat, Vikas Gupta, Addafar Gulzar, Aabid Hussain Sheikh and Syed Muzamil

Abstract
Vector-borne bacterial diseases are serious threat to several crops including apple, citrus, grapes, olive, potato, sesame, brinjal, sugarcane etc. for causing huge economical losses particularly in tropical and sub-tropical regions (Gonella *et al*., 2019). However, this pathogen is weak as its dispersal largely depends on other agents like insects, animals, planting material etc. Hemipterans play a major role in its dispersal and as a result of their extensive host range, fast reproduction, and capacity to spread a variety of plant diseases, they are more damaging pests of crops (Perilla-Henao and Casteel, 2016). The plant vascular system is enriched with nutrients that serve as a good source of food for these insects and many bacterial pathogens. With their piercing and sucking mouth parts, the hemipterans acquire pathogens by feeding on plant sap and subsequently transmit it to the healthy plant and spreading the disease. Research on vector-borne bacterial diseases is challenging because of fastidious nature of pathogen (Huang *et al*., 2020). Methods like bioinformatics, genome sequencing, transcriptomics and genetic manipulation has improved our compression, knowledge and understanding towards pathogen-plant-vector relationship to a great extent (Perilla-Henao and Casteel, 2016) [65]. As a result of this information, we can devise novel disease control measures. Endosymbionts and commensals, for example, have a role in transmission by changing acquisition, improving insect fitness, fecundity, and immunity, and some have even exhibited antagonistic effects against pathogens (Gonella *et al*., 2019). Multiple pathogens compete for space and food in a single insect body, with only one pathogen effectively transmitting. By lowering vector competence, measures such as exploiting insect microbiome, para transgenesis, and cytoplasmic incompatibility can assist to reduce disease dissemination (Trivedi *et al*., 2016).

Keywords: Acquisition, fastidious bacteria, hemipteran insects, insect microbiome, vector competence

1. Introduction
The xylem and phloem of the plant vascular system store nutrients such as carbohydrates, proteins, amino acids, fatty acids, vitamins, and minerals. Plants are autotrophs or primary producers forms the most basic and important trophic level in the ecosystem (Bove and Garnier, 2003) [12]. Plants provide food for almost all living things on the planet. Plants support microorganisms such as protozoa, bacteria, fungi, viruses, and viroid's as well as macro-organisms such as insects. It is made up of two separate host habitats for plant pathogens: phloem and xylem tissues. Phloem tissue is made up of companion cells which supplies metabolic and regulatory components to the phloem sap, as well as sieve elements that form a long-distance transport route throughout the plant (Lucas, 2006; Will *et al*., 2013) [51, 94]. Plant sap is sucked by hemipterans with piercing and sucking mouth parts. Insects and vascular-limited bacteria are thus significant threats to agricultural and horticulture crops. Because vascular restricted bacteria can't travel to healthy plants on their own, they're in danger of losing their population along with the plant. They developed to attract insects for transmission by altering the look of the plant and inviting insects to the damaged plant in order to replicate themselves. The pathogen is acquired by insects through plant sap, which they then pass to healthy plants (Purcell, 1982; Nault and Ammar, 1989; Orlowski's *et al*., 2015) [67, 4].

Plants represent the kingdom Plantae, bacteria represent the kingdom Monera, and insects represent the kingdom Animalia. The interaction between these three separate kingdoms is a tripartite in which representatives from these groups interact. Several crops, including apple, citrus, grapes, olive, potato, sesame, brinjal, sugarcane, and others, are vulnerable to vector-borne bacterial infections, which can cause significant economic losses, particularly in tropical and sub-tropical areas. However, these diseases are poor in nature since it relies heavily on other agents for transmission, such as insects, animals, and plant waste. Because of their wide

host range, speedy reproduction, and capacity to transmit a variety of plant diseases, hemipterans play a key part in its spread and are hence more damaging pests of crops (Perilla-Henao and Casteel, 2016) [65]. The vascular system of plants is abundant in nutrients that make available food for these insects as well as various bacterial pathogens. Diseases are acquired by hemipteran insects upon feeding on plant sap with their piercing and sucking mouth parts, which they then transport to healthy plants hence, spreading the disease. The fastidious nature of these pathogens makes research on vector-borne bacterial diseases difficult. *Xylella fastidiosa*, *Candidatus* Phytoplasma, *Spiroplasma* spp., *Candidatus Liberibacter* spp., and *Arsenophonus*-like bacteria are among the bacteria carried by the vector. Hemipteran insects such as Planthoppers, Treehoppers, Leafhoppers, Spittlebugs, Cicadas, Aphids, Whiteflies, and Psyllids are carriers of a number of bacterial diseases. Except *Spiroplasma* all vector borne bacteria are unculturable. It is a major limitation and backlog in understanding the exact nature of these pathogens. However, the approaches like whole genome sequencing and bioinformatics, transcriptomics and genetic manipulation are quite useful in understanding the nature of these pathogens. The interactions between pathogen and insect vector body may include insect's symbionts, commensals, antagonists, competition between pathogens inside the vector bodies. The vector borne bacteria and hemipteran insects can be studied from this perspective helps us to gain information related to all steps involved in their transmission and hence lead us to develop new strategies in order to control the disease spread (Huang *et al*., 2020) [37].

2. Importance of vector-borne bacterial diseases

Plant diseases carried by vectors have far-reaching ecological and economic implications, affecting farm profitability and forest diversity all over the world. In order to interact with their hemipteran insect vectors and plant hosts, bacterial vector-borne pathogens have evolved complex and complicated tactics. These pathogens persist in plant vascular tissue, providing a unique opportunity to uncover novel molecular pathways governing intracellular pathogenesis and contribute to the control of some of the world's most virulent emerging diseases (Huang *et al*., 2020) [37]. In recent decades, these bacterial vector-borne infections have wreaked havoc on the citrus, grape, and olive sectors. Over the last few decades, vector-borne bacteria have caused some of the most harmful plant diseases in both perennial and annual crops. For example, Citrus greening disease caused by '*Candidatus Liberibacter asiaticus*,' has quickly spread around the globe from its origins in North America. Every year, citrus greening costs producers more than $4 billion, resulting in huge economic losses as well as the loss of thousands of jobs (Gottwald, 2010) [30]. The phytoplasma disease 'lime witches broom' threatens the lime industry in the Middle East (Donkersley *et al*., 2018) [21], while Huanglongbing disease (HLB) caused by *Candidatus Liberibacter asiaticus* (CLas) has caused estimated losses of over $7.8 billion in Florida since 2007 (Court *et al*., 2018) [14]. Furthermore, for ages, coconut phytoplasmas have killed millions of palm trees and are now spreading locally (Gurr *et al*., 2016) [32]. Pierce's disease of the grapevine '*Xylella fastidiosa*' has had a considerable impact on grape output, resulting in an annual cost of almost $100 million in California alone (Tumber *et al*., 2014) [86]. Olive orchards in Europe have recently been destroyed by the new pathogen *X. fastidiosa* caused olive quick decline syndrome (Schneider *et al*., 2020) [78]. The overall economic loss to the olive industry in Italy and Spain is predicted to be up to €5.2 billion in Italy and €16.86 billion in Spain over the next 50 years if disease management measures are not implemented on time (Schneider *et al*., 2020) [78].

3. Terminologies related to vector transmission

All of the following terms are defined in terms of the virus-vector interaction. The same is true for bacteria carried by vectors.

3.1 Persistence

The vector-borne virus transmission process is based on following two criteria's: (1) the time it takes for the pathogen to contact and infect the insect vector, and (2) Time spent in the vector by the pathogen (Ng and Falk, 2006) [60]. As a result of this, these are categorized into three categories:

(1) Non-persistent: In this mode, Insect vectors hold pathogens in their stylets or alimentary canals. There was no immediate transmission of a latency pathogen. The pathogen is lost after moulting and remains virulent for a few hours, with a short acquisition feeding time (Ng and Falk, 2006; Uzest *et al*., 2007) [60, 87].

(**2) Semi-persistent:** pathogens are those that remain in the insect's alimentary canal or stomach lumen. The pathogen retains virulence for a few hours to days after losing its latency at moulting (Chen *et al*., 2011; Ng and Zhou, 2015) [61].

(3) Persistent: the pathogen infects the intestinal epithelial cell and eventually makes its way to the salivary gland. There is a latency period, and the vector is infectious until it dies. It necessitates a lengthy acquisition feeding time (Ng and Falk, 2006) [60]. *Xylella fastidiosa*, a xylem colonizer, has a semi-permanent relationship with its vectors. Phloem-limited bacteria have long-term relationships with their vectors (Redak *et al*., 2004) [73].

3.2 Location

The pathogens having persistent links are of further two types: non-circulative and circulative.

(1) Non-circulative: As part of the transmission mechanism, the pathogen does not go inside the insect body and is hence retained by the insect in the stylet or foregut region (Ng and Zhou, 2015) [61].

(2) Circulative: As part of the transmission process, the pathogen enters the body of the insect after passing through the foregut and into the intestine, in which it resides until the insect dies (Gray *et al*., 2014) [31]. *Xylella fastidiosa*, for example, is a non-circulative xylem colonizer, whereas all phloem colonizers are of circulative nature.

3.3 Replication

The ability to proliferate inside the insect body is used to classify pathogens that have circulative interactions with their vector.

(1) Non-propagative: These pathogens inside the vector, does not reproduce (Nadarasah and Stavrinides, 2011) [56].

(2) Propagative: The pathogen multiplies inside the insect vector, which acts as alternate host for the pathogen. (Nadarasah and Stavrinides, 2011) [56]. The vector borne bacteria which are propagative, can propagate intracellularly or extracellularly. For example, *Xylella fastidiosa* which propagates extracellularly is a non-circulative. All phloem colonizers propagate intracellularly. Vector generally acquires the plant pathogen by feeding on infected plants. Once within the insect body, the virus penetrates past intestinal barriers, internal organs, and visceral muscles, it can establish everywhere in the hemolymph (Hogenhout *et al*., 2008; Orlowski's *et al.*, 2015) [35]. Before the disease may be transmitted to a new plant host, the virus must move from the hemolymph to the salivary glands. Only a few plants virus groups that are vector-borne have propagative associations with vectors. Rhabdoviridae, Reoviridae, and Bunyaviridae are among these families (Hogenhout *et al*., 2008; Ammar *et al*., 2009) [4, 35].

4. Taxonomy of the Hemipteran vectors

Vectors of most phytopathogenic diseases belong to order Hemiptera, however few are from Coleoptera (Beetles), Orthoptera (Grasshoppers), Diptera (Maggot fly), Thysanoptera (Thrips) etc. Vascular limited bacteria are vectored by insects belonging to the order Hemiptera. Vector-borne bacteria rely on the members of the suborder *Auchenorrhyncha* and *Sternorrhyncha* (Bove and Garnier, 2003) [12]. Multiple superfamilies of the Euhemiptera lineage have been found to be vectors of bacterial plant diseases and viral plant pathogens (*Auchenorrhyncha*, *Coleorrhyncha*, and *Heteroptera*). Surprisingly, just a few species from each superfamily have been identified as efficient vectors for bacterial and viral plant diseases (Weintraub and Beanland, 2006) [90]. The Beet Leafhopper (*Circulifer tenellus*, Baker), for example, transmits the Beet Curly Top Virus ([BCTV], Geminiviridae) along with two bacterial diseases, caused by *Candidatus Phytoplasma trifolii* and *Spiroplasma citri* (Weintraub and Beanland, 2006) [90]. Leafhopper, planthopper, spittlebugs, froghoppers belong to *Auchenorrhyncha*. Aphids, whiteflies, scales and psyllids belong to *Sternorrhyncha*. Among these only psyllids from *Sternorrhyncha* transmits only bacteria not virus remaining others transmits only virus not bacteria (Hogenhout *et al*., 2008; Gilbertson *et al*., 2015) [35, 27].

5. Why these Bacterial pathogens are inhabitants of vascular system?

5.1 The nutrient pool

The transport pathways of the plant vascular system *i.e*., xylem and phloem are loaded with nutrients such as carbohydrates, amino acids, proteins, vitamins, lipids, and fatty acids. It provides pathogens with a variety of habitats in which they can thrive and hence live comfortably. The vascular system of plants acts as a channel between the source and the sink. It varies significantly between the xylem and the phloem (Bove and Garnier, 2003; Lough and Lucas, 2006) [12, 51].

5.1.1 Xylem

Xylem vessels, tracheid, xylem parenchyma, and xylem fiber's make up the xylem. Unlike phloem, xylem vessels have no end walls, so they are continuous from the root to the tip of the leaf. The xylem vessels are no longer alive. It is the primary carrier of water and minerals from the soil to the plant components above ground. The mode of transportation is one-way. The nutritional status of xylem is lower than that of phloem. Though pathogens such as *Xylella fastidiosa* resides in the xylem vessel (Purcell and Hopkins, 1996) [68].

5.1.2 Phloem

Sieve tube, companion cells, and phloem parenchyma make up phloem. Between two sieve tubes, there are sieve plates with perforated end walls. It is the primary transporter of food molecules generated during photosynthesis, known as photosynthates. Organic food is carried from green plant components such as leaves and stems to storage organs such as fruits, seeds, tubers, rhizomes, and bulbs. There are two modes of transportation. Unlike xylem, the cell walls here are alive. Phloem colonists include *Candidatus Phytoplasma*, *Spiroplasma* spp., *Candidatus Liberibacter* spp., and *Arsenophonus*-like bacteria (Bove and Garnier, 2003) [12].

6. Vector-Borne bacteria

All known vector-borne bacteria have biological characteristics such as plant vascular tissue specialization, vector propagation, and full reliance on their hosts that make them different from other pathogens. When the same resources have become available from the host environment, genome degradation is likely to have resulted in the loss of important metabolic pathways from the bacterial progenitor (Nadarasah and Stavrinides, 2011) [56]. *Xylella fastidiosa*, which colonizes the xylem, possesses the group's largest genome. This could be because xylem has fewer nutrients than phloem. For this bacterium to thrive in the nutrient-limited xylem, more critical metabolic pathways in the genome may be necessary. Despite their commonalities, the bacteria that rely on hemipteran vectors for transmission belong to a variety of phyla and orders. Several hemipterans are significant plant pests that propagate bacterial pathogens. Physical obstacles to insect feeding and processes that affect insect physiology and behavior are among the plant defenses against hemipterans (Painter, 1951; Kogan and Ortman, 1978) [63, 41].

6.1 Phloem-restricted vector-borne bacteria The only xylem colonizer transmission carried by a hemipteran vector is *Xylella fastidiosa*. It affects a wide range of plants and causes diseases such as Pierce's disease of grapes, citrus variegated chlorosis, almond leaf scorching, and olive leaf scorching. Simpson *et al*. (2000) [80] sequenced the entire genome because of its economic value. As a result, *Xylella* is the first plant microbe whose entire genome has been sequenced. Glassy-winged sharpshooter *Homalodisca vitripennis*, *Homalodisca coagulata*, and *Graphocephala atropunctata* are the major vectors of the *Xylella* (Chatterjee *et al*., 2008a; Backus and Morgan, 2011; Rapicavoli *et al*., 2015) [15, 4, 69]. Different research tools have been created to study *X. fastidiosa*, including *in vitro* culture techniques, transformation, and bacterial strain mutations (Killiny *et al*., 2012) [40]. Some of the genes involved in pathogenicity have been found using these techniques.

A cell-to-cell signaling sensor (RpfC) is used by *X. fastidiosa* as a negative regulator. Diffusible signaling factors (DSF) mediate the signaling system, which modulates various aspects of behavior in a population-dependent manner (i.e., quorum sensing). When a threshold concentration of DSF is achieved outside the cell, it is secreted into the extracellular environment, where it activates virulence, motility, and

biofilm formation pathways (Chatterjee *et al*., 2008b) [16].

1. The outermost layer of the Gram-negative bacteria lipopolysaccharide, is very important for pathogen to attach to the vector and therefore further transmission (Rapicovali *et al*., 2015).
2. Lipase/esterase effector LesA, an enzyme released by the type II secretion system, is implicated in phytotoxicity during the early stages of infection (Nascimento *et al*., 2016) [57].
3. For nutrition and mobility Endoglucanases, xylanases, xyloxidases, and polygalacturonases are among the enzymes used by *X. fastidiosa* to destroy the plant cell. Pathogenicity and mobility are absent in mutants with defective n-polygalacturonases enzyme synthesis (Roper *et al*., 2007) [76].

6.2 Phloem-restricted vector-borne bacteria

The majority of phytoplasma (class Mollicutes) and liberibacters (class Alpha proteobacteria) phytopathogens are vector-borne (Bressan, 2014) [12]. Quite few species of Spiroplasmas (class Mollicutes) are phytopathogens. All known phloem-limited vector-borne bacteria appear to colonize both the insect and plant hosts intracellularly, irrespective of their diversity. Bacteria enter through the intestine and circulate through body of the vector before reaching finally to salivary glands and hemolymph (Gasparich, 2010) [26]. A latent period of days to months is required before transmission to the salivary glands may take place (Thebaud *et al*., 2009).

6.2.1 Spiroplasmas

Spiroplasmas have a coiled morphology and a pili-like appendage that allows them to move in a corkscrew pattern. Pathogenic, commensal, and mutualistic interactions are all part of its interactions with plant and insect hosts (Ammar *et al*., 2004) [3]. There are three species which has reported phytopathogenic, *Spiroplasma citri* which causes citrus stubborn and its vector is beet leafhopper *Circulifer tenellus*, *S. kunkeli* causal agent of corn stunt disease of maize plant and is vectored by maize leafhopper, *Dalbulus maidis*, periwinkle yellows caused by *S. phoeniceum* in which natural vector is not reported yet, it is transmitted experimentally by *Macrosteles fascifrons.*

The path of spiroplasmas inside the insect body is as follows: after acquisition, spiroplasmas bind to a receptor on the gut lumen, enter the cell via endocytosis, enter the hemocoel via intracellular vesicular transfer, and finally reach the salivary glands via exocytosis for subsequent transmission, and so on (Gasparich, 2010) [26]. However, despite the discovery of several proteins that bind to the receptor, the receptor remains unknown. A monolayer cell culture test was utilized to identify the protein. The *Circulifer tenellus* (CT1) cell line was infected with spiroplasmas, and the cell culture was studied using electron microscopy and an immunofluorescence assay after colonization. Many potential proteins, such as P58, SARP, and the plasmid-borne protein P32, have been found as a result of this work. Spiralin is another abundant membrane protein of S. citri that has been linked to transmission. *Spiroplasma citri* mutants with lower spiralin synthesis show reduced transmission by *Circulifer haematoceps*, the vector (Gasparich, 2010) [26]. This indicates that spiralin is necessary for pathogen to interact with insect vector. Spiralin which acts as lectin binds to the glycoprotein of insect vector membrane.

6.2.2 Phytoplasmas

Pleomorphic Phytoplasmas do not have distinct forms. Phytoplasmas are difficult to cultivate. It has the shortest genome, measuring 0.7 megabytes, with a low G+C content, large quantity of repetitive sections (Kube *et al*., 2012). Phytoplasma has been assigned the temporary genus *Candidatus* Phytoplasma. So far, 33 groups and hundreds of subgroups have been identified. Phytoplasmas damage over 1000 plant species and have a large host range (Hogenhout *et al*., 2008) [35]. Chlorosis, witches' broom, virescence, phyllody, bolting, leaf reddening, declining of stem, stunting and rolling of foliage and unripe shoots and fruits of plants are common phytoplasma symptoms. Some of the pathogenicity factors of phytoplasmas are:

1. The examined genomes have at least six different types of ATP-binding cassette (ABC) transporters. ABC transporters are believed to allow nutrition and metabolite absorption from the host by transporting compounds across bacterial membranes (Wang *et al*., 2014) [88].
2. A superoxide dismutase enzyme (SOD), which may be used to counteract reactive oxygen species produced by hosts, and a protease (HflB), that acts as a virulence factor, are two other common traits. Apple proliferation pathogen *Candidatus Phytoplasma mali* (Wang *et al*., 2014) [88].
3. In the Onion Yellow Phytoplasma genome, a conserved Mollicutes adhesion motif (MAM) was discovered. P38, a potential protein, interacts with crude insect extracts and plant extracts on a weekly basis (Neriya *et al*., 2014) [59].
4. The secreted enzyme translocase Sec A, part of a type II secretion system, aids in the transport of specific proteins and acts as effectors (Bai *et al*., 2009) [5].
5. SAP effectors frequently change the function of the host by disrupting plant hormone homeostasis. The TENGU effector, for example, blocks auxin-related pathways, as a result the plant has a dwarf phenotype and is florally sterile (Minato *et al*., 2014) [54].
6. In *Arabidopsis* transgenic lines expressing SAP11, very less amount of jasmonic acid (JA) is produced, resulting in aberrant vegetative development and higher leafhopper vector fecundity on diseased plants (Lu *et al*., 2014b).
7. SAP54/PHYL attaches to floral transcription factors and incites MADS-box protein degradation. MADS-box proteins are required for the formation of floral meristems, and plants expressing SAP54/PHYL blossom improperly (Maejima *et al*., 2014) [76]
8. Internalization of the phytoplasma and transmission efficiency were lowered when the leafhopper vector was given a monoclonal anti-AMP from the *Candidatus Phytoplasma asteris Chrysanthemum* Yellows strain. As a result, anti-Amp appears to prevent bacteria from attaching to the vector gut (Rashidi *et al*., 2015) [72].

6.2.3 Liberibacter

Candidatus Liberibacter asiaticus, *Candidatus Liberibacter solanacearum*, *Candidatus Liberibacter africanus*, *Candidatus Liberibacter americanus*, *Candidatus Liberibacter europaeus*, and *Candidatus Liberibacter crescens* are six species of phloem-limited bacteria (Haapalainen, 2014) [33]. Three species of bacteria called *Candidatus Liberibacter africanus*, *Candidatus Liberibacter americanus*, and *Candidatus Liberibacter asiaticus* are responsible for Citrus greening disease, also known as

Huanglongbing (HLB) in different parts of the world (Gottwald, 2010) [30]. Liberibacters have a genome that is just about 1.2 megabytes in size. Comparative genomics has revealed a comparable gene architecture across the species, as well as evidence of horizontal gene transfers in the form of prophages integrated into genomes (Thompson *et al*., 2015) [84]. The bacteria "*Candidatus Liberibacter solanacearum*" ("*Ca*. *Liberibacter psyllaurous*") is phytopathogenic to Apiaceae and Solanaceae plants. For transmission and as alternate hosts, all four species rely on psyllid vectors (Fagen *et al*., 2014b; Haapalainen, 2014) [25, 33]. Although "*Ca*. *Liberibacter europaeus*" has been linked to psyllids, its significance as a plant pathogen has yet to be proven. Only *Liberibacter crescens* has been cultivated *in vitro* to yet, however it is neither phytopathogenic nor vector-borne (Fagen *et al*., 2014a) [24]. *Liberibacter crescens* was detected as a bacterial endophyte on papaya and has not been observed in nature since. Bacterial DNA has been identified in mealybugs, therefore non-psyllid hemipterans may be able to catch up the bacteria during feeding (Pitino *et al*., 2014) [65]. Biosynthetic genes for amino acids, carbohydrates, and nitrog enated bases are lacking in liberibacters similar to phytoplasmas, implying that they get these metabolic products from their hosts (Thompson *et al*., 2015) [84]. As a result, liberibacter genomes have a large number of ABC transporters (Lin *et al*., 2011; Yan *et al*., 2013) [94]. Active nutrient importation from phloem and insect vectors could cause nutritional imbalances, which could explain some of the foliar symptoms seen in Liberibacter-infected plants (Rashed *et al*., 2013) [70].

When comparing genomes with phytoplasma, certain pathogenicity factors are discovered. LasAI and LasII, two unique autotransporters identified in the liberibacter genome, may function as an alternative secretion pathway to the Sec-system (Hao *et al*., 2013) [34]. The NahG-like salicylate hydroxylase gene was discovered to cleave salicylates produced from SA, which is a key signaling chemical involved in plant defense against diseases and insects (Lin *et al*., 2013).

6.2.4 *Arsenophonus*-like bacteria

Arsenophonus-like bacteria started off as insect parasites and symbionts, but through time evolved into plant pathogens. *Ca*. *Phlomobacter fragriae*, which causes marginal chlorosis in strawberries and is transmitted by *Cixius wagneri*, and *Ca*. *Phytopathogenicus arsenophonus*, which produces basses richess disease in sugar beet and is transmitted by *Pentastiridius leporinus*, have been reported thus far. *Arsenophonus*-like bacteria have several characteristics that they resemble with insect symbionts, including reproductive tissue colonization and vertical transmission, lack of entomopathogenic action, high infection rate, and a life cycle focused primarily on insect hosts (Bressan *et al*., 2009) [13]. For instance, a survey was conducted of 136 arthropod species, out of which only 5% of the studied hosts are having association with *Arsenophonus* bacteria (Duron *et al*., 2008), where they might form complex associations with helpful or parasitic properties (Wilkes *et al*., 2011) [76].

7. Bacterial pathogen vector relationship

The relationship might be useful or harmful between bacterial pathogen and vector. *Diaphorina citri* infected with *Candidatus Liberibacter asiaticus* is more susceptible to insecticides and *Bactericera cockerelli* infected with *Candidatus Liberibacter solanacearum* has lower fecundity. Changes in protein related to energy metabolism, immunity, and lipid transfer occur at the site of the hemolymph (Mann *et al*., 2011). *Scaphoideus titani* infected with the 16SrV phytoplasma had a lower survival rate and laid fewer eggs than healthy *Scaphoideus titani*. *Macrosteles quadrlinieatus* infected with 16SrI phytoplasma, on the other hand, had a beneficial effect (Beanland *et al*., 2000). Insect immunity has an impact on pathogen acquisition. For example, *Diaphorina citri* nymphs are more effective at acquiring *Candidatus Liberibacter asiaticus* than adults because adults have well-developed immunity such as melanization and death of gut cell (Kruse *et al*., 2017) [43]. Main route for bacterial internalization found for plant pathogenic diseases is endo-exocytosis, which is mediated by several membrane proteins (Hogenhout *et al*., 2008) [35]. The lack of specific adhesion machinery greatly limits the vector's capacity to connect to host cells (Weintraub and Beanland, 2006) [90]. For example. Insects cannot transmit *S. citri* strains lacking adhesion-related proteins (Kruse *et al*., 2017) [43]. In rare situations, endosymbionts of insects have transitioned to a plant pathogenic lifestyle, such as *Ca*. *Arsenophonus phytopathogenicus* and *Spiroplasma* spp. Insect commensals, such as *Ca*. *Liberibacter* spp., have evolved to be plant pathogens. Many endosymbionts or commensals could become plant pathogens in the future, posing a risk (Morris *et al*., 2017; Gasparich, 2010) [26].

7.1 Multiple pathogens and competition

Multiple pathogens infect the plant phloem at the same time, and the pathogens may be related or unique and the interactions may be simple to complex. An insect feeds many plants during its lifecycle, and acquires various pathogens or strains of a pathogen. Interferential interactions prevent co-occurrence in all cases (Swisher *et al*., 2018). The following are some of the competitions:

1. Corn stunt spiroplasma (CSS) and maize bushy stunt phytoplasma (MBSP) both have the same vector, *Dalbulus maidis*. Since CSS is more competitive during the latency period and multiply rapidly, CSS suppresses MBSP (De Oliveira *et al*., 2007).
2. *Macrosteles quadrilineatus* was exposed to various strains of Aster Yellows Phytoplasma and among all only the first strain was transferred to it (Bosco and D'Amelio, 2010).
3. *Ca*. *P. asteris* and *Ca*. *P. phoenicium* were used to double-infect *Osbornellus horvathi* leafhoppers, were able to transmit the former but not the latter. (Rizza *et al*., 2016) [75]
4. Rashidi *et al*. (2014) [71] employed the leafhopper *Euscelidius variegatus* along with two distinct phytoplasmas, chrysanthemum yellows phytoplasma (CYP) and Flavescence doree phytoplasma (FDP), which were introduced to broad bean plants in an experiment to identify competition. The vector was exposed to CYP and FDP in particular order. The unilateral interference was observed as FDP was repressed by CYP regardless of the sequence. Competition occurs in salivary gland and CYP multiplies faster than FDP. As CYP is having long co-evolutionary history with host and hence they are able to mitigate the immune response. Analysis by the molecular techniques like transcriptomics confirmed that infection by FDP in insects induced the immune response, but CYD enhanced the energy metabolism of insects.

7.2 Symbiont pathogen interaction

Both facultative and obligate endosymbionts reside inside insect body and the role of obligatory and facultative endosymbionts in insects is to provide nutrition and maintain host fitness (Baumann *et al*., 2005) [15]. '*Ca*. Sulcia muelleri' which belongs to subgroup Auchenorrhyncha and '*Ca*. Carsonella ruddii' from psyllids are the predominant obligate (primary) symbionts. To integrate its food source with the insect, Sulcia also requires additional (co-primary) symbiotic bacteria (McCutcheon & Moran, 2010) [53]. *Wolbachia*, *Cardinium*, *Rickettsia*, and *Arsenophonus* are some of the reproductive manipulators (Iasur-Kruh *et al*., 2017) [37]. Here are a few examples:

1. *Diaphorina citri* has three endosymbionts: *Wolbachia*, *Carsonella ruddi*, and *Ca.* Profftella armatura. *Ca.* Profftella armatura synthesises two proteins: diaphorin and diaphorin-like polyketides and provides protective function while as *Ca*. Carsonella ruddi necessities nutritional advantages. In *Ca*. *Liberibacter asiaticus* infected vectors, diphorin synthesise is downregulated, but two proteins involved in polyketide biosynthesis have higher levels (Ramsey *et al*., 2015) [68].
2. Dyella-like bacteria from the Xanthomonadaceae family were isolated from *Hyalesthus obsoletus* and found to have anti-phytoplasma activity. It demonstrated endophytic features in wild shrub *Vitex agnus-castus* while being isolated from insects. When inoculated into the grape plant, the pathogen was immediately inhibited, which reduced disease symptoms (Isaur-Kruh *et al*., 2018).
3. Bacteria (Asaia) that make acetic acid decreased FDP acquisition from broad bean was isolated from mosquitoes orally consumed into the vector *E. Variegatus*. Asaia affects FDP's capacity to pass intestinal epithelia (Crotti *et al*., 2009) [18].
4. Because *Nausia* is an obligatory symbiont of leafhoppers that transmit phytoplasma and is absent in non-vector leafhoppers, *Nausia* appears to be necessary for effective transmission (Wangkeeree *et al*., 2012) [89].
5. The plant hopper vector of FDP, *Dictyophora europea*, revealed a negative link between phytoplasma and *Wolbachia* (Krstić *et al*., 2018).

8. Methodologies for studying bacteria carried by vectors

Methods like genome sequencing, bioinformatics, transcriptomics and genetic manipulation has improved our understanding towards pathogen-plant-vector interactions to some extent (Perilla-Henao and Casteel, 2016) [65]. The ability to grow vector-borne bacteria has a big influence on our present understanding of pathogenicity mechanisms. Only *X. fastidiosa* and *Spiroplasma* spp. have been cultivated *in vitro* to date, and both require extremely precise and definite conditions (Dourado *et al*., 2015) [22]. Many of the processes and biology of phytoplasma and liberibacter host colonization are unknown as a result of this limitation (Bove and Garnier, 2003) [12]. Another concern is that phloem-limited vector-borne bacteria are spread non-homogeneously, making tissue selection for the purpose of sampling challenging. Another issue is the generation of distinct symptoms in different plants.

8.1 Whole genome sequencing and Bioinformatics for vector-borne bacteria

The genome sequences of several vector-borne bacteria have recently been revealed, making them easily accessible now. This has made scientists easy to investigate the function of bacterial genes in these systems without having to cultivate the organism. Bioinformatics can be used to correlate genome sequences to annotated genomes of distant relatives, or to evaluate sequences using server-based algorithms to allocate predicted functions to each coding region (Rutherford *et al*., 2000) [77]. Amino acid patterns can be examined further to reveal conserved patterns and domains. Proteins having a low average similarity can be allocated to a projected function in this way. Finally, all patterns *viz.*, signal peptides, localization, cleavage sites, phosphorylation, and transmembrane domain patterns are all recognized by specialized algorithms and can be used to predict the activities of unknown proteins (Yu *et al*., 2010).

One drawback of these many bioinformatics approaches is that they all require cultured organisms to train their programme's. Several unique sequences exist for unculturable bacteria that have no homologs in cultured species, making comparisons and conclusions difficult. Despite these shortcomings, bioinformatics has been successfully explored to investigate the function of number of phytoplasma effector genes (Bai *et al*., 2009) [5].

8.2 Transcriptomics of vector-borne bacteria

Functional validation is essential when putative pathogenicity factors have been discovered. Only the transcription and translation of targets may be examined inside the host in unculturable bacteria. RNA and protein should be extracted for this purpose. However, phytoplasma RNA is only recovered in a small percentage of total RNA taken from infected herbaceous hosts; just 0.1 per cent of total RNA isolated from infected herbaceous hosts contains phytoplasma RNA. Similarly, phytoplasma RNA from the woody host accounts for only 0.02 per cent of the total (Abba *et al*., 2014). High-throughput sequencing, on the other hand, has opened up new avenues for researching infections inside their hosts. RNAseq may now be used to quantify a sample's whole RNA population (Westermann *et al*., 2012) [91]. Compared to microarrays and qRT-PCR, the approach has a higher sensitivity for monitoring gene expression levels, is not limited to studying just known sequences, and has a larger detection range. *Candidatus* Phytoplasma mali transcription in tobacco was investigated using RNAseq (*Nicotiana occidentalis*). Plant ribosomal depletion kit is used to enrich phytoplasma RNA. Only 0.003 per cent of the total reads were mapped to the anticipated *Candidatus* Phytoplasma mali genome's protein coding regions. Out of 497 predicted genes, 132 were mapped (Siewert *et al*., 2014; Abba *et al*., 2014) [79].

8.3 Genetic manipulation of vector-borne bacterial phytopathogens

The use of transposon mutagenesis with *Spiroplasmas* was the first method for discovering gene functions for vector-borne bacterial plant diseases. *Spiroplasma citri* chromosome were randomly integrated with a transposon containing a selection marker, and recombinant colonies were selected in antibiotic-containing media. When transformed colonies were examined in the host, the transposon was kept for a few days without antibiotic pressure. This method was employed to show that disrupting a solute binding protein in the leafhopper vector hindered transmission (Boutareaud *et al*., 2004) [10]. Gene function for other vector-borne bacteria has been tested through genetic modification employing surrogate culturable

bacteria and heterologous gene expression in plants (Jain *et al*., 2015) [39]. Overexpression of bacterial candidate proteins in the plant host is an alternate way for researching gene function. Plant pathogen gene function is commonly assessed using model plants such as *Arabidopsis thaliana* and *Nicotiana* spp. The coding sequence of the selected gene is then cloned into appropriate expression vector, and transgenic plants can be developed. Several scientists have examined the function of phytoplasma SAPs using plant heterologous expression systems. During these studies, transgenic *A. thaliana* expressing individual phytoplasma SAPs was examined for symptom development and plant sufferings (Lu *et al*., 2014a; Yang *et al*., 2015) [95]. Changes in plant gene expression and plant proteins, interaction with phytoplasma proteins were investigated if a relevant phenotype was established. The fact that only substantial abnormalities produced by a single bacterial gene may be recognized is a limitation of this method. Furthermore, model plants may not be natural hosts for all vectored-borne bacteria, limiting the applicability of findings to an artificial system.

8.4 Control strategies for the future

We can build some unique tactics to control the spread of vector-borne bacteria using the knowledge gathered from the preceding procedures. Insecticides are currently employed on a massive basis to control the insect vector however they are insufficient and unsustainable. Insecticide use may result in some serious problems such as the development of insect resistance and polluting of the environment. Another option is to use host plant resistance; this is a good option, but it will take a long time to identify resistant resources and build a resistant variety, therefore it is not feasible (Bisognin *et al*., 2008; Riaz *et al*., 2008) [8, 74]. We can apply innovative tactics such as inhibiting pathogen adhesion to the insect vector by using chemicals. In the insect, for example, lectin competes with bacteria for binding sites. *X. fastidiosa* adhesions on the cell surface are saturated by N acetyl glucosamine carbohydrates. The goal of this procedure is to saturate the insect's pathogen binding sites. *Eucelideus variegatus* was fed a meal plus bacteria plus transmission-blocking substances. Under greenhouse conditions, the pathogen transmission was reduced and hence indicative results are obtained (Killiny *et al*., 2012) [40].

Another method is the gene drive system, which uses the naturally occurring phenomena selfish DNA, a genetic element found in all human genomes that has the ability to replicate itself. Identifying a vector symbiont and putting selfish DNA designed with an antipathogenic gene into the vector to provide antipathogenic protein, suppressing the pathogen and limiting transmission. Transgenic *Wolbachia* is an example of this method (Sinkins and Gould, 2006) [81]. Another option is the use of RNAi, which is widely used in the treatment of viral diseases. By changing reproduction, physiology, and survival, RNAi can be utilized to control insect vectors. Direct injection, bait feeding, and transgenic plants are all approaches for inducing RNAi in insects. Because direct injection and bait feeding are not viable options. As a result, transgenic plants are the greatest option for employing RNAi to control bacterial pathogen vectors (Gordon and Waterhouse, 2007; Donald *et al*., 2012; Li *et al*., 2013; Yu *et al*., 2016) [29, 20].

9. Harnessing microbiome for vector control

As a result of this information, we can devise unique disease control measures. Endosymbionts and commensals, for example, have a role in transmission by changing acquisition, improving insect fitness, fecundity, and immunity, and some have even exhibited antagonistic effects against pathogens (Gonella *et al*., 2019) [28]. Understanding the microbial ecology and functionality in a certain habitat is referred to as Microbial Research Management (MRM). The Asian citrus psyllid is inhibited by a conidial-based formulation of *Metarhizium anisopliae*, *Isaria fumosorosea*, and *Hirstuella citriformis* that was developed to suppress pathogens or lower vector competence. Para transgenesis and cytoplasmic incompatibility are two methods for preventing the transmission of the infection by vector. Another technique is to boost the insect's immunity. Volatile-mediated deterrent, as we all know, is beneficial to insects in locating their host. We can prevent insects from reaching their host by altering their volatile profile (Trivedi *et al*., 2016) [85].

10. Conclusion and future considerations

These hemipteran vectors' transmission mechanisms could be persistent, propagative, or circulative, and they are mostly members of the suborders Auchenorrhyncha and Sternorrhyncha. A tiny subset of known vector-borne bacteria, including organisms from many phylogenetic kingdoms, may reproduce in both the plant host and the insect vector. Several guests are vying for the same insect vector, which complicates study. Endosymbiotic, commensalistic, or antagonistic relationships exist between insects and pathogens. We may utilize current technologies like whole genome sequencing and bioinformatics, transcriptomics, RNAi, Gene Driven Systems, and chemical transmission inhibition to analyze and manage these vector-borne-bacteria. More attention should be paid to research on vector-borne diseases. Only a few insects vector-pathogen relationships have been investigated so far. These relationships could be beneficial to diverse. It should be intensified and hence made available to everyone for further studies. Developing adequate *in vitro* growth techniques and consolidating genomic databases for all vector-borne bacteria should be emphasized. In the future, endosymbionts could become a pathogen, so it becomes necessary to keep an eye on this situation. Because pathogens are not evenly distributed in the host, the sampling material for early detection should be standardized for each crop. Because symptoms manifest slowly, strategies to detect the presence of a pathogen in an insect vector should be devised. Agricultural diseases spread by bacterial vectors are among the most economically significant and widespread. The study of these pathologies is poised to find new biological mechanisms due to their unusual biological characteristics, which include colonization of both host vascular tissue and the insect vector. We hope that the concepts offered in this perspective will inspire future generations of scientists to look into the numerous open problems about vector-borne diseases and contribute to the future.

11. References

1. Abbà S, Galetto L, Carle P, Carrère S, Delledonne M, Foissac X. RNA-Seq profile of flavescence dorée phytoplasma in grapevine. BMC genomics. 2014;15(1):1-13.
2. Ammar ED, Fulton D, Bai X, Meulia T, Hogenhout SA. An attachment tip and pili-like structures in insect-and plant-pathogenic spiroplasmas of the class Mollicutes.

Archives of microbiology. 2004;181(2):97-105.
3. Ammar ED, Tsai CW, Whitfield AE, Redinbaugh MG, Hogenhout SA. Cellular and molecular aspects of rhabdovirus interactions with insect and plant hosts. Annual review of entomology. 2009;54:447-468.
4. Backus EA, Morgan DJ. Spatiotemporal colonization of *Xylella fastidiosa* in its vector supports the role of egestion in the inoculation mechanism of foregut-borne plant pathogens. Phytopathology. 2011;101(8):912-922.
5. Bai X, Correa VR, Toruño TY, Ammar ED, Kamoun S, Hogenhout SA. AY-WB phytoplasma secretes a protein that targets plant cell nuclei. Molecular Plant-Microbe Interactions. 2009;22(1):18-30.
6. Baumann P. Biology of bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annual Review of Microbiology. 2005;59:155-189.
7. Beanland L, Hoy CW, Miller SA, Nault LR. Influence of aster yellows phytoplasma on the fitness of aster leafhopper (Homoptera: Cicadellidae). Annals of the Entomological Society of America. 2000;93(2): 271-276.
8. Bisognin CL, Schneider B, Salm H, Grando MS, Jarausch W, Moll E. Apple proliferation resistance in apomictic rootstocks and its relationship to phytoplasma concentration and simple sequence repeat genotypes. Phytopathology. 2008;98(2):153-158.
9. Bosco DD, Amelio R. Transmission specificity and competition of multiple phytoplasmas in the insect vector. *Phytoplasmas* (PG Weintraub and P. Jones Eds.) CAB International, Wallingford. UK. 2010, 293-308.
10. Boutareaud A, Danet JL, Garnier M, Saillard C. Disruption of a gene predicted to encode a solute binding protein of an ABC transporter reduces transmission of *Spiroplasma citri* by the leafhopper *Circulifer haematoceps*. Applied and environmental microbiology. 2004;70(7):3960-3967.
11. Bove JM, Garnier M. Phloem-and xylem-restricted plant pathogenic bacteria. Plant Science. 2003;164:423-438.
12. Bressan A. Emergence and evolution of *Arsenophonus* bacteria as insect-vectored plant pathogens. Infection. Genetics and Evolution. 2014;22:81-90.
13. Bressan A, Arneodo J, Simonato M, Haines WP, Boudon-Padieu E. Characterization and evolution of two bacteriome-inhabiting symbionts in cixiid planthoppers (Hemiptera: Fulgoromorpha: Pentastirini). Environmental microbiology. 2009;11(12):3265-3279.
14. Court C, Hodges A, Rahmani M, Spreen T. Economic Contributions of the Florida Citrus Industry in. 2018, 2015-16: FE1021, 7/2017. EDIS. 2.
15. Chatterjee S, Almeida RPP, Lindow S. Living in two worlds: the plant and insect lifestyles of *Xylella fastidiosa*. Annual Review of Phytopathology. 2008a;46:243-271.
16. Chatterjee S, Wistrom C, Lindow SE. A cell–cell signaling sensor is required for virulence and insect transmission of *Xylella fastidiosa*. Proceedings of the National Academy of Sciences. 2008b;105(7):2670-2675.
17. Chen AY, Walker GP, Carter D, Ng JC. A virus capsid component mediates virion retention and transmission by its insect vector. Proceedings of the National Academy of Sciences. 2011;108(40):16777-16782.
18. Crotti E, Damiani C, Pajoro M, Gonella E, Rizzi A, Ricci I. Asaia, a versatile acetic acid bacterial symbiont, capable of cross-colonizing insects of phylogenetically distant genera and orders. Environmental microbiology. 2009;11(12):3252-3264.
19. De Oliveira E, Santos JC, Magalhaes PC, Cruz I. Maize bushy stunt phytoplasma transmission by *Dalbulus maidis* is affected by spiroplasma acquisition and environmental conditions. Bulletin of insectology. 2007;60(2):229.
20. Donald CL, Kohl A, Schnettler E. New insights into control of arbovirus replication and spread by insect RNA interference pathways. Insects. 2012;3(2):511-531.
21. Donkersley P, Blanford JM, Queiroz RB, Silva FW, Carvalho CM, Al-Sadi AM. Witch's broom disease of lime (*Candidatus* Phytoplasma aurantifolia): Identifying high-risk areas by climatic mapping. Journal of economic entomology. 2018;111(6):2553-2561.
22. Dourado MN, Santos DS, Nunes LR, Costa de Oliveira RLBD, de Oliveira MV, Araujo WL. Differential gene expression in *Xylella fastidiosa* 9a5c during co-cultivation with the endophytic bacterium *Methylobacterium mesophilicum* SR1. 6/6. Journal of basic Microbiology. 2015;55(12):1357-1366.
23. Duron O, Bouchon D, Boutin S, Bellamy L, Zhou L, Engelstädter J. The diversity of reproductive parasites among arthropods: Wolbachiado not walk alone. BMC biology. 2008;6(1):1-12.
24. Fagen JR, Leonard MT, Coyle JF, McCullough CM, Davis-Richardson AG, Davis MJ. *Liberibacter crescens* gen. nov., sp. nov., the first cultured member of the genus Liberibacter. International journal of systematic and evolutionary microbiology. 2014a;64(7):2461-2466.
25. Fagen JR, Leonard MT, McCullough CM, Edirisinghe JN, Henry CS, Davis MJ. Comparative genomics of cultured and uncultured strains suggests genes essential for free-living growth of Liberibacter. PLoS One. 2014b;9(1):84469.
26. Gasparich GE. Spiroplasmas and phytoplasmas: microbes associated with plant hosts. Biologicals. 2010;38(2):193-203.
27. Gilbertson RL, Batuman O, Webster CG, Adkins S. Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. Annual review of virology. 2015;2:67-93.
28. Gonella E, Tedeschi R, Crotti E, Alma A. Multiple guests in a single host: interactions across symbiotic and phytopathogenic bacteria in phloem-feeding vectors-a review. Entomologia Experimentalis et Applicata. 2019;167(3):171-185.
29. Gordon KH, Waterhouse PM. RNAi for insect-proof plants. Nature biotechnology. 2007;25(11):1231-1232.
30. Gottwald TR. Current epidemiological understanding of citrus huanglongbing. Annual review of phytopathology. 2010;48:119-139.
31. Gray S, Cilia M, Ghanim M. Circulative, nonpropagative virus transmission: an orchestra of virus-, insect-, and plant-derived instruments. In Advances in virus research. Academic Press. 2014;89:141-199.
32. Gurr GM, Johnson AC, Ash GJ, Wilson BA, Ero MM. Coconut lethal yellowing diseases: a phytoplasma threat to palms of global economic and social significance. Frontiers in plant science. 2016;7:1521.
33. Haapalainen M. Biology and epidemics of *Candidatus* Liberibacter species, psyllid-transmitted plant-pathogenic bacteria. Annals of applied biology. 2014;165(2):172-198.

34. Hao G, Boyle M, Zhou L, Duan Y. The intracellular citrus Huanglongbing bacterium, '*Candidatus Liberibacter asiaticus*' encodes two novel autotransporters. PLoS One. 2013;8(7):68921.
35. Hogenhout SA, Ammar ED, Whitfield AE, Redinbaugh MG. Insect vector interactions with persistently transmitted viruses. Annual Review of Phytopathology. 2008;46:327-359.
36. Huang W, Reyes-Caldas P, Mann M, Seifbarghi S, Kahn A, Almeida RP. Bacterial vector-borne plant diseases: unanswered questions and future directions. Molecular plant. 2020;13(10):1379-1393.
37. Iasur-Kruh L, Naor V, Zahavi T, Ballinger MJ, Sharon R, Robinson WE. Bacterial associates of *Hyalesthes obsoletus* (Hemiptera: Cixiidae), the insect vector of bois noir disease, with a focus on cultivable bacteria. Research in microbiology. 2017;168(1):94-101.
38. Iasur-Kruh L, Zahavi T, Barkai R, Freilich S, Zchori-Fein E, Naor V. Dyella-like bacterium isolated from an insect as a potential biocontrol agent against grapevine yellows. Phytopathology. 2018;108(3):336-341.
39. Jain M, Fleites LA, Gabriel DW. Prophage-encoded peroxidase in '*Candidatus Liberibacter asiaticus*' is a secreted effector that suppresses plant defenses. Molecular Plant-Microbe Interactions. 2015;28(12):1330-1337.
40. Killiny N, Rashed A, Almeida RP. Disrupting the transmission of a vector-borne plant pathogen. Applied and Environmental Microbiology. 2012;78(3):638-643.
41. Kogan M, Ortman EF. Antixenosis-a new term proposed to define Painter's nonpreference modality of resistance. Bulletin of the ESA. 1978;24(2):175-176.
42. Krstić O, Cvrković T, Mitrović M, Radonjić S, Hrnčić S, Toševski I. Wolbachia infection in natural populations of *Dictyophara europaea*, an alternative vector of grapevine Flavescence doree phytoplasma: effects and interactions. Annals of Applied Biology. 2018;172(1):47-64.
43. Kruse A, Fattah-Hosseini S, Saha S, Johnson R, Warwick E, Sturgeon K. Combining'omics and microscopy to visualize interactions between the Asian citrus psyllid vector and the Huanglongbing pathogen *Candidatus Liberibacter asiaticus* in the insect gut. PloS one. 2017;12(6):0179531.
44. Kube M, Mitrovic J, Duduk B, Rabus R, Seemüller E. Current view on phytoplasma genomes and encoded metabolism. The Scientific World Journal. 2012.
45. Li J, Wang XP, Wang MQ, Ma WH, Hua HX. Advances in the use of the RNA interference technique in Hemiptera. Insect Science. 2013;20(1):31-39.
46. Lin H, Coletta-Filho HD, Han CS, Lou B, Civerolo EL, Machado MA. Draft genome sequence of *Candidatus Liberibacter americanus* bacterium associated with citrus huanglongbing in Brazil. Genome announcements. 2013;1(3):00275-13.
47. Lin H, Lou B, Glynn JM, Doddapaneni H, Civerolo EL, Chen C. The complete genome sequence of '*Candidatus Liberibacter solanacearum*', the bacterium associated with potato zebra chip disease. PLoS One. 2011;6(4):19135.
48. Lu YT, Cheng KT, Jiang SY, Yang JY. Post-translational cleavage and self-interaction of the phytoplasma effector SAP11. Plant Signaling & Behavior. 2014a;9(6):28991.
49. Lu YT, Li MY, Cheng KT, Tan CM, Su LW, Lin WY. Transgenic plants that express the phytoplasma effector SAP11 show altered phosphate starvation and defense responses. Plant physiology. 2014b;164(3):1456-1469.
50. Lucas WJ. Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. Virology. 2006;344:169-184.
51. Maejima K, Iwai R, Himeno M, Komatsu K, Kitazawa Y, Fujita N. Recognition of floral homeotic MADS domain transcription factors by a phytoplasmal effector, phyllogen, induces phyllody. The Plant Journal. 2014;78(4):541-554.
52. Mann RS, Pelz-Stelinski K, Hermann SL, Tiwari S, Stelinski LL. Sexual transmission of a plant pathogenic bacterium, *Candidatus Liberibacter asiaticus*, between conspecific insect vectors during mating. PLoS One. 2011;6(12):29197.
53. McCutcheon JP, Moran NA. Functional convergence in reduced genomes of bacterial symbionts spanning 200 My of evolution. Genome biology and evolution. 2010;2:708-718.
54. Minato N, Himeno M, Hoshi A, Maejima K, Komatsu K, Takebayashi Y. The phytoplasmal virulence factor TENGU causes plant sterility by downregulating of the jasmonic acid and auxin pathways. Scientific reports. 2014;4(1):1-7.
55. Morris J, Shiller J, Mann R, Smith G, Yen A, Rodoni B. Novel '*Candidatus* Liberibacter'species identified in the Australian eggplant psyllid, *Acizzia solanicola*. Microbial Biotechnology. 2017;10(4):833-844.
56. Nadarasah G, Stavrinides J. Insects as alternative hosts for phytopathogenic bacteria. FEMS microbiology reviews. 2011;35(3):555-575.
57. Nascimento R, Gouran H, Chakraborty S, Gillespie HW, Almeida-Souza HO, Tu A. The type II secreted lipase/esterase LesA is a key virulence factor required for *Xylella fastidiosa* pathogenesis in grapevines. Scientific reports. 2016;6(1):1-17.
58. Nault LR, Ammar ED. Leafhopper and planthopper transmission of plant viruses. Annual review of entomology. 1989;34(1):503-529.
59. Neriya Y, Maejima K, Nijo T, Tomomitsu T, Yusa A, Himeno M. Onion yellow phytoplasma P38 protein plays a role in adhesion to the hosts. FEMS Microbiology Letters. 2014;361(2):115-122.
60. Ng JC, Falk BW. Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. Annual Review of Phytopathology. 2006;44:183-212.
61. Ng JC, Zhou JS. Insect vector-plant virus interactions associated with non-circulative, semi-persistent transmission: Current perspectives and future challenges. Current opinion in virology. 2015;15:48-55.
62. Orlovskis Z, Canale MC, Thole V, Pecher P, Lopes JR, Hogenhout S.A. Insect-borne plant pathogenic bacteria: getting a ride goes beyond physical contact. Current Opinion in Insect Science. 2015;9:16-23.
63. Painter RH. Insect resistance in crop plants. 1951;72(6):481. LWW.
64. Perilla-Henao LM, Casteel CL. Vector-borne bacterial plant pathogens: interactions with hemipteran insects and plants. Frontiers in Plant Science. 2016;7:1163.
65. Pitino M, Hoffman MT, Zhou L, Hall DG, Stocks IC, Duan Y. The phloem-sap feeding mealybug (*Ferrisia virgata*) carries '*Candidatus Liberibacter asiaticus*' populations that do not cause disease in host plants. PLoS

One. 2014;9(1):85503.
66. Purcell AH. Insect vector relationships with prokaryotic plant pathogens. Annual Review of Phytopathology. 1982;20:397-417.
67. Purcell AH, Hopkins DL. Fastidious xylem-limited bacterial plant pathogens. Annual review of phytopathology. 1996;34(1):131-151.
68. Ramsey JS, Johnson RS, Hoki JS, Kruse A, Mahoney J, Hilf ME. Metabolic interplay between the Asian citrus psyllid and its Profftella symbiont: an Achilles' heel of the citrus greening insect vector. PLoS One. 2015;10(11):0140826.
69. Rapicavoli JN, Kinsinger N, Perring TM, Backus EA, Shugart HJ, Walker S, Roper MC. O antigen modulates insect vector acquisition of the bacterial plant pathogen *Xylella fastidiosa*. Applied and Environmental Microbiology. 2015;81(23):8145-8154.
70. Rashed A, Wallis CM, Paetzold L, Workneh F, Rush CM. Zebra chip disease and potato biochemistry: tuber physiological changes in response to '*Candidatus Liberibacter solanacearum*' infection over time. *Phytopathology*. 2013.103(5): 419-426.
71. Rashidi M, D'amelio R, Galetto L, Marzachì C, Bosco D. Interactive transmission of two phytoplasmas by the vector insect. Annals of Applied Biology. 2014;165(3):404-413.
72. Rashidi, M., Galetto, L., Bosco, D., Bulgarelli, A., Vallino M, Veratti F, Marzachì C. Role of the major antigenic membrane protein in phytoplasma transmission by two insect vector species. BMC microbiology. 2015;15(1):1-12.
73. Redak RA, Purcell AH, Lopes JR, Blua MJ, Mizell Iii RF, Andersen PC. The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. Annual Reviews in Entomology. 2004;49(1):243-270.
74. Riaz S, Tenscher AC, Rubin J, Graziani R, Pao SS, Walker MA. Fine-scale genetic mapping of two Pierce's disease resistance loci and a major segregation distortion region on chromosome 14 of grape. Theoretical and Applied Genetics. 2008;117(5):671-681.
75. Rizza S, Pesce A, D'Urso V, Raciti E, Marzachì C, Tessitori M. Transmission of '*Candidatus* Phytoplasma asteris' (16SrI) by Osbornellus horvathi (Matsumura 1908) co-infected with '*Ca*. Phytoplasma phoenicium' (16SrIX). Phytoparasitica. 2016;44:491-500.
76. Roper MC, Greve LC, Warren JG, Labavitch JM, Kirkpatrick BC. *Xylella fastidiosa* requires polygalacturonase for colonization and pathogenicity in *Vitis vinifera* grapevines. Molecular Plant-Microbe Interactions. 2007;20(4):411-419.
77. Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream MA. Artemis: sequence visualization and annotation. Bioinformatics. 2000;16(10):944-945.
78. Schneider K, Van der Werf W, Cendoya M, Mourits M, Navas-Cortés JA, Vicent A. Impact of *Xylella fastidiosa* subspecies *pauca* in European olives. Proceedings of the National Academy of Sciences. 2020;117(17):9250-9259.
79. Siewert C, Luge T, Duduk B, Seemüller E, Büttner C, Sauer S. Analysis of expressed genes of the bacterium '*Candidatus* Phytoplasma mali'highlights key features of virulence and metabolism. PloS one. 2014;9(4):94391.
80. Simpson AJG, Reinach FDC, Arruda P, Abreu FAD, Acencio M, Alvarenga R. The genome sequence of the plant pathogen *Xylella fastidiosa*. Nature. 2000;406(6792):151-157.
81. Sinkins SP, Gould F. Gene drive systems for insect disease vectors. Nature Reviews Genetics. 2006;7(6):427-435.
82. Swisher KD, Munyaneza JE, Velásquez-Valle R, Mena-Covarrubias J. Detection of pathogens associated with psyllids and leafhoppers in *Capsicum annuum* L. in the Mexican states of Durango, Zacatecas, and Michoacan. Plant disease. 2018;102(1):146-153.
83. Thébaud G, Yvon M, Alary R, Sauvion N, Labonne G. Efficient transmission of '*Candidatus* Phytoplasma prunorum'is delayed by eight months due to a long latency in its host-alternating vector. Phytopathology. 2009;99(3):265-273.
84. Thompson SM, Johnson CP, Lu AY, Frampton RA, Sullivan KL, Fiers MW. Genomes of '*Candidatus Liberibacter solanacearum*' haplotype A from New Zealand and the United States suggest significant genome plasticity in the species. Phytopathology. 2015;105(7):863-871.
85. Trivedi P, Trivedi C, Grinyer J, Anderson IC, Singh BK. Harnessing host-vector microbiome for sustainable plant disease management of phloem-limited bacteria. Frontiers in Plant Science. 2016;7:1423..
86. Tumber KP, Alston JM, Fuller K. Pierce's disease costs California $104 million per year. California Agriculture. 2014;68:1-2.
87. Uzest M, Gargani D, Drucker M, Hébrard E, Garzo E, Candresse T. A protein key to plant virus transmission at the tip of the insect vector stylet. Proceedings of the National Academy of Sciences. 2007;104(46):17959-17964.
88. Wang Q, Guo Y, Wang N, Li Y, Chen W, Chen W. Identification of a conserved core genome with group-specific genes from comparative genomics of ten different *Candidatus* Phytoplasma strains. Journal of Phytopathology. 2014;162(10):650-659.
89. Wangkeeree J, Miller TA, Hanboonsong Y. Candidates for symbiotic control of sugarcane white leaf disease. Applied and Environmental Microbiology. 2012;78(19):6804-6811.
90. Weintraub PG, Beanland L. Insect vectors of phytoplasmas. Annual Review of Entomology. 2006;51:91-111.
91. Westermann AJ, Gorski SA, Vogel J. Dual RNA-seq of pathogen and host. Nature Reviews Microbiology. 2012;10(9):618-630.
92. Wilkes T, Duron O, Darby AC, Hypša V, Nováková E, Hurst GDD. The genus Arsenophonus Manipulative tenants: bacteria associated with arthropods (ed. by E Zchori-Fein & K Bourtzis). CRC Press, Boca Raton, Fl, USA. 2011, 225–244.
93. Will T, Furch AC, Zimmermann MR. How phloem-feeding insects face the challenge of phloem-located defence's. Frontiers in Plant Science. 2013;4:336.
94. Yan Q, Sreedharan A, Wei S, Wang J, Pelz-Stelinski K, Folimonova S. Global gene expression changes in *Candidatus Liberibacter asiaticus* during the transmission in distinct hosts between plant and insect. Molecular plant pathology. 2013;14(4):391-404.
95. Yang CY, Huang YH, Lin CP, Lin YY, Hsu HC, Wang CN, Liu LYD. MicroRNA396-targeted Short Vegetative Phase is required to repress flowering and is related to the

development of abnormal flower symptoms by the phyllody symptoms1 effector. Plant Physiology. 2015;168(4):1702-1716.
96. Yu NY, Wagner JR, Laird MR, Melli G, Rey S, Lo R. PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics. 2010;26(13):1608-1615.
97. Yu XD, Liu ZC, Huang SL, Chen ZQ, Sun YW, Duan PF. RNAi-mediated plant protection against aphids. Pest Management Science. 2016;72(6):1090-1098.