IN SEARCH OF INSECT VECTORS OF PHYTOPLASMAS WITH A NOTE ON VECTORS OF XYLELLA FASTIDIosa

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ABSTRACT

Phytoplasmas are phloem restricted, Gram positive bacteria without cell walls and are transmitted by phloem-sap feeding Hemiptera families such as Cicadellidae, Delphacidae, Cixiidae, Derbidae, Psyllidae and rarely by Pentatomidae and Tingidae. In the first part, this review briefly presents information available on the interaction among the phytoplasmas, their insect vectors, host plants and also very briefly the environment. In the second part, it deals with the xylem restricted, vector transmitted, Gram negative, Xylella fastidiosa (Wells et al.) and most likely candidate vectors if the organism gets accidentally introduced into India. Two proven vectors of X. fastidiosa in Taiwan namely, Kolla paulula (Walker) and Bothrogonia ferruginea (Fabricius) occur in India. In the third part, ways to search for the promising vectors of both the pathogens is dealt with. Emphasis is laid on for a close cooperation amongst insect taxonomists, molecular biologists and plant pathologists to look into investigations dealing with these organisms right from the initial stages. Brief account of confirmed and putative vectors of phytoplasmas in India and most likely groups of Auchenorrhyncha that may vector X. fastidiosa are given and the species dealt with are illustrated for ease of preliminary identification. Use of recent advances in tissue culture, vector biology, artificial feeding of Hemiptera and molecular biology are suggested for fast tracking the search for vectors of these pathogens.

Key words: Aphrophoridae, Auchenorrhyncha, Cercopidae, Cicadellidae, Delphacidae, Derbidae, Hishimonus, Orosius, phytoplasma, vector-pathogen interaction, Xylella fastidiosa

Ever increasing number of plant diseases caused by phytoplasmas both in cultivated and wild plants are being reported every year. There are more than 700 phytoplasma diseases so far reported, many of them lethal in hundreds of plant species in the world (Weintraub and Beanland, 2006). Phytoplasmas being confined to a particular plant tissue depend largely on insects for transmission from infected to a healthy plant. Though insects remain the most important vectors of these organisms, unfortunately, information on them as vectors of phytopathogens in general and phytoplasmas in particular is very scanty. For many phytoplasma diseases present in India and known for more than a century such as sandal spike disease, coconut root (wilt) and areca yellow leaf diseases, information on their confirmed vectors is not available and only putative vectors are known.

There are several recent reviews on phytoplasma vectors by several workers namely Purcell (1982), Waintraub and Beanland (2006), Bosco and D’Amelo (2010), Alma et al. (2019) and Weintraub et al. (2019) and also on Xylella fastidiosa and its vectors by Redak et al. (2004), Almeida et al. (2005), Purcell (2013) and Sicard et al. (2018). This paper heavily draws information from these reviews.

Phytoplasmas

Phytoplasmas are phloem restricted, insect transmitted phytopathogenic, Gram positive prokaryotes (bacteria without cell wall), closely related to mycoplasmas and spiroplasmas. They are obligate parasites and techniques available so far have failed to grow them in culture in artificial media. They are however, capable of multiplying both in their plant hosts and insects that transmit them. They interfere with the physiology of plants and modify plant morphology by stunting their growth, reducing leaf size, shortening internodal length and converting floral parts into either vegetative parts or make them sterile. Phytoplasmas can also kill the infected plants, as in the case of sandal spike, in a few years after infection. Both monocotyledonous and dicotyledonous plants, wild or cultivated, are affected by phytoplasmas and some infected plant species may
serve as symptomless carriers of these organisms and others as dead-end hosts.

Being phloem tissue bound, they depend on either procedures used in vegetative propagation of plants such as cuttings, grafting etc., or through stolon, rhizomes and bulbs for their spread. A few phytoplasmas are transmitted through seeds as in the case of phytoplasmas affecting alfalfa, carnation, tomato, and citrus (Khan et al., 2002; Botti and Bertaccini, 2006; Satta et al., 2019) and coconut lethal yellowing (Cordova et al., 2003). Phytoplasmas can also be transmitted through the parasitic plants of the genus Cuscuta.

Phytoplasmas move in host plant through sieve tube elements in the phloem (Christensen et al., 2004). Often they are distributed unevenly in the host plant tissues. There is also seasonal fluctuation in the titre of phytoplasma in woody plants. Generally, level of phytoplasmas is very low in the roots (sink) and moderate in stems. The highest titre was found in source organs (mature leaves, sometimes almost 40 times higher than that in roots). In sink leaves (very young leaves) the titre remains low or below detectable levels. In deciduous plants the phytoplasma might disappear from the aerial parts of trees during winter, and survive in the root system to recolonize the aerial parts during spring. However, there are several exceptions for this phenomenon and may depend on the phytoplasma and the plant species involved. In some cultivated fruit trees it may be found in scions, and also in aerial parts in winter (Bertaccini and Duduk, 2009).

Taxonomy of leafhopper vectors

Hemiptera are the fifth most speciose order of insects with more than 103590 valid species known from the world. Three of the four suborders of Hemiptera, Auchenorrhyncha, Heteroptera and Sternorrhyncha host the confirmed vectors. The families Cicadellidae (81% of the known phytoplasma vector species), Cixiidae (6%), Delphacidae (4%), Derbidae (1%) Flatidae (1%), Issidae (1%) are among Auchenorrhyncha; the families Pentatomidae (1%) and Tingidae (1%) are among Heteroptera and Psyllidae (7%) among Sternorrhyncha contain phytoplasma vectors.

Among these groups the family Cicadellidae (leafhoppers) contain most of the vectors. Many leafhoppers are phloem feeders thus forming a pool of potential vectors for phloem limited phytoplasmas (Weintraub et al., 2019). Classification of this family based on phylogenetic analysis using morphological and molecular characters recognises 19-20 subfamilies

(Dietrich et al., 2001; Zahniser and Dietrich, 2008). The most highly derived lineage of the leafhopper subfamily Deltocephalinae contains more than 75% of all confirmed phytoplasma leafhopper vectors in the world. The feeding habits of Deltocephaline leafhoppers range from monophagous to polyphagous and members can transmit one or more phytoplasma taxa. The tribe Macropsini of the subfamily Eurymelinae contains the second largest number of confirmed phytoplasma vectors and is also fairly highly derived lineage. Macropsini are also either monophagous or polyphagous and feed primarily on woody plants with a few exceptions feeding on annual herbs. Membracidae are phylogenetically closely related to Cicadellidae, however none of them are at present known as phytoplasma vectors. They feed on woody plants gregariously and exhibit sub-social behaviour. It would not be surprising to find that they transmit phytoplasmas primarily associated with woody plants.

Mouthparts and feeding behaviour of phytophagous Hemiptera

For efficient transmission phytoplasmas need a vector that picks them up from the phloem tissue of the infected plant and introduces into phloem tissue of a healthy susceptible plant without causing much tissue damage. This needs highly specialized organisms equipped with structures which can extract nutrients from vascular bundles with least destruction to the host tissue.

Hemiptera have highly specialized suctorial mouthparts to extract sap from different tissues without harming the plant tissue. The suctorial proboscis consists of a stylet bundle capable of piercing intact tissue of plants such as leaf lamina to hard seeds, roots to bark. The stylet bundle consists of four very fine highly chitinized yet supple and innervated needles. The outer pair, derived from mandibles, can move independently and are adorned with concentric ridges on outer surface of each stylet near apex to anchor the stylet bundle. The inner pair, derived from maxillae are held together tightly by interlocking ridges and grooves and are channelled to house dorsal food canal and ventral much narrower salivary duct and both meet near the tip of the stylet into one canal. The labium is segmented, flexible and surrounds the stylet bundle. A group of highly specialized chemoreceptors present at the tip of the labium help to select the host plant and locate the surface to probe. The labium does not take part in piercing which is done by the stylet bundle. The labrum is a short, triangular lobe at the base on dorsum.
of the labium and is ventrally indented to direct and position the stylet bundle. At the base of each stylet there are levers on which the muscles are attached which effect movement of each stylet. As the leafhoppers and planthoppers feed, their stylet bundle is surrounded by a sheath formed by viscous saliva secreted by the anterior part of the salivary glands which solidifies on exposure to outer environment and protects the stylets. The phytoplasmas and other circulatory pathogens are introduced into the phloem probably along with watery saliva (secreted by the posterior part of the salivary gland) as the leafhopper stylets penetrate sieve element membranes (Lett et al., 2001).

The food canal posteriorly leads to precibereum which also has chemoreceptors and also a preciberial valve that probably regulates the flow of the fluid and probably also prevents unwanted outward flow of ingested sap. The precibereum leads to the cibereum which has very strong musculature to pump the sap into the osphagus (ciberial pump) (Backus, 1985). This pump is very strongly developed in leafhoppers that feed on xylem vessels (where the xylem sap is under negative pressure) compared to those feeding on phloem sap (which is under 2-3 times the atmospheric pressure). The strong muscles required to pump the xylem sap need to be housed on the head region and hence the xylem feeding leafhoppers have greatly swollen faces as in the case of Cicadellinae, Evacanthinae and Signoretiiinae.

Among the Hemiptera only those that feed on phloem tissues can efficiently transmit the phytoplasmas. These include the members of the families Cicadellidae, Delphacidae, Cixiidae, Issidae, Pentatomidae and Tingidae. The insect vectors (both suspected and proven) reported from India are listed in Table 1 and their photographs are also given for ease of identification (Figs. 1-36).

Hemiptera show some plasticity in feeding on specific host plant tissue. Phloem, xylem and mesophyll feeding guilds are not strict categories, and especially among vascular feeding leafhoppers the distinction between the phloem feeding and xylem feeding guilds is blurred (Wayanade, 1994) and feeding preference could depend on the host plant (resistant and susceptible) and gender (Chuche et al., 2017). Therefore, although phloem feeding behaviour is a pre-requisite for transmission of phytoplasmas and other phloem-limited phytopathogens, one cannot exclude the possibility that species feeding primarily on xylem transmit phytoplasmas. Typhlocybinae leafhoppers are mesophyll feeders but some species may also feed on phloem sap and may be less efficient transmitters of phytoplasmas compared to specialist phloem feeding leafhoppers.

A vector may be able to acquire specific phytoplasma from one plant species but not from another or may acquire a phytoplasma from one plant species but may not be able to transmit to a different species (Bosco et al., 1997). *Empoasca decipiens* Paoli could acquire Chrysanthemum Yellows Phytoplasma (*Ca. P. asteris*, 16SrI-B) (CYP) and transmit to *Ismelia carinata* (Schousb.) Sch.Bip. (as *Chrysanthemum carinatum* Schousb.) but is not able to acquire from or transmit the same phytoplasma to broad bean (*Vicia faba* L.) (Galetto et al., 2011).

**Phytoplasma insect vector interactions**

The terminologies used to describe various interactions between phytoplasmas and their vectors are same as those used for interaction between the phytopathogenic viruses and their insect vectors.

**Acquisition and transmission**

Phloem feeding insects acquire phytoplasmas passively during feeding in the phloem of the infected plant. The Acquisition Access Period (AAP) is the feeding duration necessary to acquire a sufficient titre of phytoplasma. The AAP could vary from a few minutes to several hours and longer the AAP, the greater the chance of acquisition (Purcell, 1982). The AAP may depend on several factors such as titre of the phytoplasma, vector species involved, age and temperature etc. It usually ranges from a few days to more than 80 days (Nagaich et al., 1974; Murral et al., 1996). During the latent period the phytoplasmas pass through midgut, circulate in the haemolymph and infect malpighian tubules, fat bodies, brain (Lherminier et al., 1990; Lefol et al., 1994; Nakashima and Hayashi, 1995) and even the reproductive organs (Kawakita, 2000). The flavescence doree phytoplasma binds strongly to the tissues of alimentary canal and salivary glands but not to the muscles or genital organs of its vector, *Scaphoideus titanus* Ball (Lefol et al., 1993).

The biochemical and molecular factors involved
<table>
<thead>
<tr>
<th>Vector species</th>
<th>Disease association/phytoplasma group</th>
<th>Host plant</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>Nephotettix nigropictus</em> (Stål)</td>
<td>Rice yellow dwarf/ 16SrXI-B</td>
<td>Rice</td>
<td>Nasu et al. (1967)</td>
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<td><em>Nephotettix virescens</em> (Distant)</td>
<td>Rice yellow dwarf/ 16SrXI-B</td>
<td>Rice</td>
<td>Nasu et al. (1967)</td>
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<td></td>
<td>Sandal spike</td>
<td>Sandal tree</td>
<td>Shivararamakrishnan and Sen-Sarma (1978)</td>
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<tr>
<td><em>Exitianus indicus</em> (Distant)</td>
<td>Sugarcane grassy shoot</td>
<td>Sugarcane</td>
<td>Rao et al. (2014)</td>
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<tr>
<td><em>Deltiocephalus vulgaris</em> Dash and Virakamath</td>
<td>Sugarcane grassy shoot disease</td>
<td>Sugarcane</td>
<td>Singh et al. (2002)</td>
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<td><em>Maiestas dorsalis</em> (Motschulsky)</td>
<td>Rice orange leaf disease</td>
<td>Coconut</td>
<td>Kumara et al. (2015)</td>
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<tr>
<td><em>Maiestas portica</em> (Melichar)</td>
<td>Sugarcane grassy shoot</td>
<td>Sugarcane</td>
<td>Tiwari et al. (2017)</td>
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<td><em>Matsumuratettix hiroyophilicus</em> (Matsumura)</td>
<td>White leaf phytoplasma / 16SrXI-B</td>
<td>Sugarcane</td>
<td>Matsumoto et al. (1970)</td>
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<td><em>Cofana unimaculata</em> (Signoret)</td>
<td>Weligama coconut leaf wilt disease</td>
<td>Coconut</td>
<td>Kumara et al. (2015)</td>
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<td><em>Kolla cyelonica</em> (Melichar)</td>
<td>Weligama coconut leaf wilt disease</td>
<td>Coconut</td>
<td>Kumara et al. (2015)</td>
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<td><em>Neoaliturus fenestratus</em> (Herrich-Schäffer)</td>
<td>Phyllyody</td>
<td>Compositae</td>
<td>Klein (1970)</td>
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<td><em>Neoaliturus tenellus</em> (Baker)</td>
<td>Beet leafhopper transmitted virecence disease</td>
<td>Vegetables</td>
<td>Oldfield et al. (1977)</td>
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<td></td>
<td>Tomato big bud/ 16SrVI-A, 16Sr-IX</td>
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<td>Shaw et al. (1993), Gopalaand Rao (2018)</td>
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<td>Weintraub et al. (2004)</td>
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<td><em>Hishimonus phycitis</em> (Distant)</td>
<td>Eggplant little leaf</td>
<td>Eggplant</td>
<td>Mararosch et al. (1970)</td>
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<td>16SrI-B, 16SrII-D</td>
<td>Parthenium, Cannabis, Jasmine, Chrysanthemum</td>
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<td>Sesame</td>
<td>Kersing and Baspinar (1997)</td>
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<td>Alfalfa</td>
<td>Salehi et al. (1995)</td>
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<td>Potato</td>
<td>Nagaich et al. (1974)</td>
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<td>Solanaceae</td>
<td>Weintraub et al. (2004)</td>
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<td><em>Orosius albicinctus</em> Distant</td>
<td>Sesamum phyllyody</td>
<td>Sesame</td>
<td>Kersing and Baspinar (1997)</td>
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<td></td>
<td>Leucern Witches’-broom</td>
<td>Alfalfa</td>
<td>Salehi et al. (1995)</td>
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<td>Purple top</td>
<td>Potato</td>
<td>Nagaich et al. (1974)</td>
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<td>16Sr-V, 16Sr-IX</td>
<td>Solanaceae</td>
<td>Weintraub et al. (2004)</td>
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<td>Limonium phytoplasma</td>
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<td><em>Idioscopus clypealis</em> (Lethierry)</td>
<td>Weligama coconut leaf wilt disease</td>
<td>Coconut</td>
<td>Kumara et al. (2015)</td>
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<td><em>Yamatotettix fulvivittatus</em> Matsumura</td>
<td>Sugarcane white leaf phytoplasma</td>
<td>Sugarcane</td>
<td>Hanboonsong et al. (2006)</td>
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<td><em>Oldiana kirkaldyi</em> (Nielson)</td>
<td>Sandal spike</td>
<td>Sandal tree</td>
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<td>misidentified as <em>Jassus indicus</em></td>
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<td><em>Alebroides nigroscutellatus</em> (Distant)</td>
<td>Potato purple top-roll/ 16SrIII-B</td>
<td>Potato</td>
<td>Singh and Nagaich (1977)</td>
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<td><em>Amrasca devastans</em> (Distant)</td>
<td>Eggplant little leaf</td>
<td>Solanaceae</td>
<td>Maramarosch et al. (1970)</td>
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<tr>
<td><em>Mesargas albomaculatus</em> (Distant)</td>
<td>Sandal spike</td>
<td>Sandal tree</td>
<td>Dover and Appanna (1934)</td>
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<tr>
<td><em>Nilaparvata lugens</em> (Stål)</td>
<td>Un-named phytoplasma</td>
<td>Rice</td>
<td>Cook and Perfect (1989)</td>
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<td><em>Redarator himaculatus</em> Distant</td>
<td>Sandal spike</td>
<td>Sandal tree</td>
<td>Ghosh et al. (1983), Balasundaran et al. (1988)</td>
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<td><em>Halyomorpha halys</em> Stål</td>
<td>Arecanut yellow leaf</td>
<td>Arecanut</td>
<td>Ponnammam et al. (1991)</td>
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<td><em>Stephanitis typica</em> (Distant)</td>
<td>Spear rot disease of oil plam</td>
<td>Oil palm</td>
<td>Kochu Babu (1993)</td>
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<td>Weligama coconut leaf wild disease</td>
<td>Coconut</td>
<td>Kumara et al. (2015)</td>
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<td></td>
<td>Paulownia Witches’-broom</td>
<td>Paulownia sp.</td>
<td>Hiruki (1999)</td>
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<td>Coconut Root wilt</td>
<td>Coconut</td>
<td>Mathen et al. (1990)</td>
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<td>Wiligama coconut leaf wilt disease</td>
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Figs. 18-29. Hemipteran vectors of phytoplasmas in India and other countries. 18. Neoaliturus fenestratus (Herrich-Scaefler); 19. Matsumuratettix hieroglyphicus (Matsumura); 20. Olidiana kirkaldyi (Nielson); 21. Amrasca devastans (Distant); 22. Mesargus albomaculatus (Distant); 23-25. Nilaparvata lugens (Stål), male, female and part of hind leg, respectively; 26-27. Redarator bimaculatus Distant, habitus dorsal view and face, respectively; 28. Halyomorpha picus (Fabricius); 29. Stephanitis typica (Distant)
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Figs. 30-36. Hemipteran vectors of phytoplasmas in their natural habitat. 30. *Oliidianna kirkaldyi* (Nielsen); 31. *Exitianus indicus* (Distant); 32. *Deltiocephalus vulgaris* Dash and Viraktamath; 33. *Mesargus albomaculatus* (Distant); 34. *Proutista moesta* (Westwood); 35. *Nephotettix virescens* (Distant); 36. *Hishimonus phycitis* (Distant)
in the movement of the phytoplasma are unknown. However, a non-insect transmissible strain of onion yellow phytoplasma had much smaller genomic size (870kbp) compared to the insect transmitted wild type (1000kbp) suggesting that the mechanism of binding to insect cells of the strain has been lost (Oshima et al., 2001). Phytoplasmas possess a major antigenic membrane protein (AMP) that make up majority of their cell surface proteins that interact with microfibrils of the intestinal muscles of the insects. The protein is believed to be an important factor for transmission and infection (Suzuki et al., 2006; Hoshi et al., 2007). Once the phytoplasma enters the intestine through the food canal of the insect mouthparts, they pass through the intestine and are absorbed into the haemolymph, subsequently the salivary glands are colonised (Bertaccini and Duduk, 2009). The phytoplasma may overwinter in insect vectors or in the perennial plants and may interact with host tissues which may improve or affect their fitness (Christensen et al., 2005). Some phytoplasmas are trans-ovarially transmitted for example, S. cynosbati and aster yellows Phytoplasma (AYP) (Danielli et al., 1996; Alma et al., 1997); Hishimimonoides sellatiformis Ishihara and mulberry dwarf (Kawakita et al., 2000); Matsumuratettix hiroglyphicus (Matsumura) and sugarcane white leaf (Honboonsong et al., 2002); Yamatotettix flavovittatus Matsumura and sugarcane white leaf (Hanboonsong et al., 2006); Cacopsylla melannoeura Forster and apple proliferation phytoplasma (Tedeschi et al., 2006).

The resistance of the salivary glands to phytoplasma infection may be one of the reasons why some species acquire phytoplasmas but are not vectors. The phytoplasma must pass through specific cells of salivary glands and accumulate in high titre in posterior acinar cells before it can be transmitted. At each point the pathogen has to overcome barriers posed by the host tissue cells, if it fails to overcome these, the phytoplasma cannot be transmitted (Klien et al., 1998; Wallace and Murphy, 1938; Weintraub et al., 2004) and the insect becomes a dead-end host (a plant that can be inoculated and subsequently become infected with the phytoplasma but from which insects cannot acquire the phytoplasma). The barriers posed by the salivary glands are basal lamina, the basal plasmalemma and the apical plasmalemma (Wayadande et al., 1997). Individuals in a population of vector species may vary in their efficiency of transmission of phytoplasmas (Galetto et al., 2009).

Host range of phytoplasmas is strongly dependent on the insect vectors as they depend on their vectors for spread. It is also possible that the host range of phytoplasma may be much wider than the combined host range of its insect vectors. Sometimes an insect can transmit a given phytoplasma under laboratory conditions, but under natural conditions they are not vectors as they do not feed on all the natural host plants of the phytoplasma. E. variegatus a laboratory vector of flavescence doree (FDP) on broad bean is not the natural vector of grapevine FDP.

Some phytoplasma vector leafhoppers can also transmit other pathogens like viruses, rickettsia-like organisms and spiroplasmas. For example, Neoaliturus tenellus (Baker) transmits beet curly top hybrid gemini virus (Wallace and Murphy 1938), phytoplasma (Weintraub et al., 2004) and Spiroplasma citri (Klein et al., 1988) but whether they use same receptor sites in the midgut cells or different ones is not known (Weintraub and Beanland, 2006).

There are complex biological interactions between the phytoplasmas and the insect vector that transmit them. There appears to be high degree of fidelity between the vector species and the phytoplasmas they transmit. However, several phytoplasmas like aster yellows and Western X strains in North America are transmitted by several species of vectors (Ebbert et al., 2001). Similarly, a single vector may transmit two or more phytoplasma strains (Lee et al., 1996).

Vector host plant interactions also play an important role in determining the spread of the phytoplasma. Polyphagous vectors have the potential to infect a wide range of plant species. It is also shown that insects that normally do not feed on certain plant species can acquire from and transmit phytoplasmas to those plants under laboratory conditions.

Bosco et al. (1997) showed that the leafhoppers differ in their ability to acquire the phytoplasmas from different infected plant species. Chrysanthemum yellows (CYP) is successfully transmitted by three leafhoppers, E. variegatus, Macrosteles quadripunctulatus Kirschbaum and Euscelis incises Kirschbaum. All three species can acquire phytoplasma from infected chrysanthemum and transmit it to uninfected chrysanthemum. However, only M. quadripunctulatus and E. variegatus could acquire phytoplasma from CYP infected periwinkle and subsequently transmit to healthy plants. On the other hand, none of the leafhoppers could acquire the phytoplasma from CYP infected celery, a dead-end host. Several dead-end hosts are known for the phytoplasmas, for example for AYP potato and cyclamen; for Western X...
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X disease peach.

The mechanisms that prevent phytoplasma acquisition from dead-end plants are not well understood. The absence of phytoplasmas may be one of the factors in some plant parts. The phytoplasmas may not be found in leaves that were mature before infection (Siddique et al., 1998). Leafhopper behaviour also provides explanations as leafhoppers alter feeding behaviour depending on the plant host (Backus et al., 2005) and this change may influence the titre of the ingested phytoplasma or ingestion of phytoplasmas. *Nephotettix virescens* (Distant) feeds principally from phloem in rice but occasionally consumes some xylem sap. However, in virus resistant cultivars, it feeds primarily on xylem (Khan and Saxena, 1984). Leafhoppers do not feed in the phloem readily on non-preferred host plants (Chiyakowski and Sinha, 1988). This may explain why only some plants are phytoplasma acquisition hosts. Phytoplasmas also change the entire physiology of the host thus affecting the normal nutritional and hormonal balance in the plant tissues making them either attractive or unattractive to the feeding vector. This may also affect the acquisition of the phytoplasma by the vector. Phytoplasma may not also be there in symptomatic plant parts (Weintraub and Beanland, 2006).

There are no reports on the vectors selectively acquiring one phytoplasma from a host plant infected with more than one phytoplasma strains. *M. quadrilineatus* could acquire and subsequently transmit a single strain when given short AAP on lettuce. This is however, a result of short AAP rather than selective acquisition or transmission (Zhang et al., 1998).

Factors mediating vectors capacity

Leafhopper gender can influence the acquisition and transmission dynamics of phytoplasmas. Female *M. quadrilineatus* were more efficient at transmitting AYP to lettuce than males (Beanland et al., 1999). However, Lefol et al. (1994) observed that the phytoplasma titre was lower in males at an earlier age compared to at an older age in the salivary glands of *E. varieigatus* compared to those in females. Behaviourally also the males are more active and move around more in search of mate compared to the females.

Vector age is another important factor influencing acquisition and transmission of phytoplasmas. Neonate *E. varieigatus* do not acquire CYP with the same efficiency as the fifth instar nymphs (Palerno et al., 2001). Efficiency of transmission is increased when they acquire the pathogen as nymphs rather than as adults (Murral et al., 1996).

Effect of phytoplasmas on its leafhopper vectors

Phytoplasmas are obligate endosymbionts with a reduced genome and lacking genes required for major metabolic processes (Oshima et al., 2004). They rely on their insect vectors for dispersal and may influence the vector’s biology, behaviour directly once infected and indirectly through the host plant. Phytoplasmas manipulate insect and plant host in their favour by suppressing host immune responses, altering host cell processes and interfering with plant development.

Direct effects

They may directly influence the vector’s biology: influence the abundance of their populations, improve fitness of the vector by increasing survival rates, life span and fecundity. Sometimes the phytoplasma may affect the vector adversely. For example, the FDP infected *S. titanus* have reduced survival and reduced fecundity, this may be due to the recent association of these two organisms. FDP infected *S. titanus* disperses less compared to uninfected individuals. On the other hand, AYP infected *M. quadrilineatus* displayed greater mobility compared to the uninfected individuals. Some phytoplasmas may not have any effect on their vectors.

Indirect effects

The phytoplasmas may manipulate insect vector behaviour through infected host plants. They can change the volatile composition of the host plant thus making the host plant more attractive to the vector. They may also change the leaf area reflectiveness. They may alter plant nutrient quality and suppress the herbivore induced defence responses to promote insect feeding and oviposition. *M. quadrilineatus* laid more eggs and produced higher number of nymphs on aster yellows-witches’ broom infected *Arabidopsis thaliana* (L.) Heynh. plants.

Epidemiology of phytoplasma diseases

Several phytoplasmas that are there in the host plants may not be of importance in a geographical area because of their inability to spread without an efficient vector. The vector and the phytoplasmas might not have come together in their evolutionary sojourn, but when they come together the phytoplasma disease may cause severe economic loss in plants affected by it. The FDP was there in Europe as a very minor pathogen until *S. titanus* got introduced into Europe during 1950s
resulting in epidemic spread of FDP in the vineyards of southern Europe. Similarly, *E. variegatus*, a Palearctic leafhopper introduced into North America, became a vector of Western X-disease and AYP.

Host range of both phytoplasma and its vector greatly influence chances that a phytoplasma and a potential vector will come into contact. Species of *Cacopsylla* are monophagous on pome or stone fruits and hence are the only vectors of ‘*Candidatus Phytoplasma mali*’, ‘*Ca. Phytoplasma pyri*’ and ‘*Ca Phytoplasma prunorum*’ which infect pome or stone fruits (Weintraub and Beanland, 2006). *Orosius albicinctus* Distant, a polyphagous leafhopper on herbaceous hosts (Suryanarayana et al., 1998) transmits different species/strains of phytoplasmas to several plant species (Table 1).

Bosco and D’Amelio (2010) recognised four categories of phytoplasma and vector relationships based on their specificity which profoundly influences the epidemiology of the disease.

**Phytoplasma and vector relationships and phytoplasma epidemiology**

**Plant generalist and vector generalist**

The phytoplasmas infect several species of plant hosts and use leafhoppers that feed on several species of plant hosts. Some examples are aster yellows transmitted by several vector species to hundreds of plant species (Weintraub and Beanland, 2006). Western X disease ‘*Ca. Phytoplasma pruni*’ (Lee et al., 2000).

**Plant generalist and vector specialist**

The beet leafhopper transmitted virescence (BLT) has several host plant species but is transmitted only by *N. tenellus*.

**Plant specialist and vector generalist**

Some phytoplasmas can be transmitted by a narrow range of vector species but some plant specialists are known to be transmitted by different vector species. Maize bushy stunt is transmitted by *Dalbulus maidis* DeLong, *D. eliminate* (Ball) and *Graminellani grifrons* (Forbes). Similarly, apple proliferation phytoplasma is transmitted by *Cacopsylla costalis* (Flor), *C.melanoeura* and *Fieberiella florii* (Stål).

**Plant specialist and vector specialist**

Some plant specialist phytoplasmas are transmitted by a specific vector only. Grapevine FDP is transmitted only by *S. titanus* in nature.

Epidemiologically transmission cycle could be grouped either as closed cycle or open cycle. In a closed cycle, the phytoplasma circulates between a main, if not exclusive, host plant and a main, if not exclusive, vector species as in grapevine FDP and its vector *S. titanus*. In an open cycle, the phytoplasma circulates among different host plants as its vector(s) can regularly or accidentally, feed on different plant species (crops). Bois Noir (BN) in grapevines is transmitted by *Hyales theses obsoletus* Signoret from weeds to grapevine. Peach Western X disease is transmitted by *Paraphlepsius irroratus* (Say) and other species from Chokecherry to peach.

**Phytoplasma vectors of the Indian subcontinent**

In this section, information on the confirmed as well as putative vectors of phytoplasmas and *Xylella fastidiosa* are given briefly. The confirmed vectors of phytoplasma include two species of *Nephotteix* Ishihara, *O. albicinctus* and *Hishimonus phycitis* (Distant). All others are here considered as putative until further confirmations are published. Several reviews are available on the phytoplasma diseases associated with plants in India namely, sandal spike (Teixeir da Silva et al., 2016), little leaf of brinjal (Rao and Kumar, 2017), sesamum phyllody (Rao et al., 2015), coconut root (wilt) phytoplasma (Rajan, 2011) and sugarcane grassy stunt phytoplasma (Rao et al., 2014).

**Species of *Nephotteix***: The genus belongs to the tribe Chiasmini and the genus *Exitianus* Ball is a close relative. They are grass feeders. *N. nigropictus* (Stål) (Figs. 2, 3) and *N. virens* (Figs. 4, 5, 35) are the species of green leafhoppers on rice which are confirmed vectors of rice yellow dwarf (Table 1) in India and abroad. Both *N. nigropictus* and *N. virens* breed on species of the genus *Oryza* and nymphs develop on that host and the former species can also breed on grasses. However, adults can be found on a number of hosts both dicots and monocots. Considerable information on their biology, ecology and management has accumulated as they are considered serious pests of rice. *N. virens* occurs in two morphs namely, green and blue morphs (Muniyappa and Viraktamath, 1981) and both are efficient vectors. The third species *N. malayanus* Ishihara and Kawase (Fig. 1) is listed as a vector of yellow dwarf (Wilson and Turner, 2010).

**Hishimonus phycitis** (Distant): The genus *Hishimonus* includes very similar looking species
feeding on herbs and shrubs. The genus has been recently revised and includes 23 species from the Indian subcontinent (Viraktamath and Anantha Murthy, 2014; Meshram and Chaubey, 2016). The genus belongs to the tribe Opsini that includes most important vectors of plant viruses, phytoplasmas and spiroplasmas (Nielson, 1979). *H. phycitis* (Figs.16, 36) is the most common species on shrubs and herbs both in cultivated and uncultivated areas. This and also *O. albicinctus* can be collected together on *Parthenium hysterophorus* L. and sesame. It is a polyphagous species using several plant species for oviposition and nymphal development. The adults feed on a number of plants other than those on which the nymphs develop (Bindra and Sohi, 1968; Bindra and Singh, 1969). The leafhopper breeds well on brinjal, *Amaranthus mangostanus* L., *A. tricolor* L., *Lepidium sativum* L., *Lime* (*Citrus aurantifolia* (Christm.) Swingle), desi cotton (*Gossypium arboretum* L.) and sesame (Bindra and Singh, 1969). The species is distributed in the Oriental region and in the Middle East in the Palaearctic region.

**Orosius albicinctus** Distant: *O. albicinctus* (Fig. 17) also belongs to the tribe Opsiini and is one of the most important leafhopper vectors transmitting a number of phytoplasmas to different crops in India and abroad (Table 1). It is a species distinct from *O. orientalis* (Matsumura) with which Ishihara (1982) had erroneously synonymised it. Fletcher et al. (2017) revised the genus based on male genitalia and molecular characters and correctly reinstated *O. albicinctus* as a valid species. They also mentioned that the populations found in India, Pakistan, Middle East and North Africa belong to this species. It is a polyphagous species using a number of herbaceous plants as oviposition and nymphal development hosts (Suryanarayana et al., 1998) whereas the adults feed on a number of plants belonging to several plant families. *Trigonella foenum-graecum* L., *Parthenium* and *Crotalaria juncea* L. are good hosts for rearing this species in the laboratory apart from sesame.

**Neoaliturus tenellus** (Baker) and *N. fenestratus* (Herrick-Schäffer): The beet leafhopper, *Circulifer tenellus* (Baker) is now placed in the Opsini genus *Neoaliturus* Distant and the Indian species *Neoaliturus gardineri* (Distant) described from Minicoy, Lakshadweep has been synonymised with the Palaearctic species *N. fenestratus* (Herrick-Schäffer) (Fig. 18). These are well-known vectors of a number of plant viruses, phytoplasmas and spiroplasmas (Table 1). Ironically, in India none of them have been incriminated as vectors. Bindra et al. (1970) reported the occurrence of *N. tenellus* (Fig. 15), *N. dubius* (Matsumura) and *N. opacipennis* (Lethierry) from northwest India (Punjab, Haryana, Himachal Pradesh and Rajasthan). *N. opacipennis* is very common in northwest India including Delhi breeding on Chenopodiaceae plants whereas *N. tenellus* is very rare. Bindra et al. (1970) collected these leafhoppers on several species of plants however; nymphs and adults of *N. opacipennis* were collected on *Heliotropium eichwaldi* Steud. and *Peganum harmala* L. and those of *N. tenellus* on *Eruca sativa* (Miller) Thell. and *Depterigerium* sp. *N. fenestatus* occurs in Minicoy (Lakshadweep Islands) and also in Ullal (Karnataka) along the sea coast and breeds on a species of the genus *Launaea* (Asteraceae). These species need to be included in transmission studies on suspected phytoplasma and spiroplasma diseases to evaluate their role as vectors.

**Exitianus indicus** (Distant): This is a leafhopper of the tribe Chiasmini mainly breeds on grasses. It is very widely distributed in the Indian subcontinent. *E. indicus* (Figs. 6, 31) is suspected as a vector of sugarcane phytoplasma (Table 1). However, it failed to transmit the white leaf sugarcane phytoplasma in Thailand (Hanboonsong et al., 2002, 2006).

**Species of the tribe Deltoccephalini**: Species of the genera *Deltoccephalus* Burmeister, *Maiestas* Distant and *Matsumuratettix* Ishihara belong to this tribe and contain either proven or putative vectors (Table 1). *Deltoccephalus vulgaris* Dash & Viraktamath (Figs. 7, 32) is reported as a vector of sugarcane grassy shoot phytoplasma (Table 1). It mainly breeds on grasses and attains large populations. It occurs in two forms as far as the male genitalia is concerned (Dash and Viraktamath, 1998) and both the morphs in the same population have been proved to belong to the same species molecularly (Zhang et al., 2019). Zig zag leafhopper, *Maiestas dorsalis* (Motschulsky) (Fig. 8) (earlier placed in the genera *Deltoccephalus*, *Inazuma* Ishihara and *Recilia* Edwards) breeds on rice and vectors a number of viruses and phytoplasma affecting rice. However, no reports of these diseases are there in India. *Maiestas portica* (Melichar) is again a polymorphic species with respect to the development of black spot on crown and coloration of head and pronotum varying from golden yellow to dull creamy white without black spot (Fig. 9). It breeds on grasses in moist habitats and has wide distribution in the Oriental region. It has been associated with transmission of sugarcane phytoplasma (Table 1). *M. hieroglyphicus* (Fig. 19) earlier known as...
Pruthiorosius maculatus (Pruthi) in India is a proven vector of sugarcane white leaf phytoplasma in Thailand and the phytoplasma is trans-ovarially transmitted (Hanboonsong et al. 2002). It is a very rare species in India and should be searched in areas surrounding sugarcane fields. It breeds on grasses. It can be readily identified by its reticulate forewing venation, however there are couple of species of Maestas resembling this species externally but have entirely different male genitalia.

Goniagnathus punctifer (Walker): This species belongs to the tribe Goniagnathini and breeds on grasses and feeds both on grasses and dicotyledonous herbs. G. punctifer (Fig. 10) vectors Weligama coconut leaf wilt phytoplasma in Sri Lanka (Table 1).

Thomsonia porrecta (Walker): This grass feeding leafhopper is common throughout the Oriental region. It is suspected as a vector of phytoplasma disease (Wilson and Turner, 2010). T. porrecta (Fig. 14) was earlier placed in the genera Hecalus Stal and Thomsoniella Signoret. It is a common species on grasses and also on rice.

Cicadelline genera Cofana Melichar and Kolla Distant: These genera belong to the subfamily Cicadellinae and the tribe Cicadellini. They are mainly xylem feeders. Kumara et al. (2015) incriminated Cofana unimaculata (Signoret) and Kolla ceylonica (Melichar) as the putative vectors of Wilegama coconut leaf wilt phytoplasma along with G. punctifer. C. unimaculata (Fig. 11) is commonly found on rice ecosystem and also on seedlings of various Poaceae crops such as wheat, ragi (Elusine corocana Gaertn.) etc. K. ceylonica (Fig. 12) is found on grasses and also on dicotyledonous herbs and shrubs. It would be of great interest if they prove to be efficient vectors of the phytoplasma.

Idioscopus clypealis (Lethierry): This is one of the most important species of mango leafhoppers belonging to the subfamily Euymelinae. I. clypealis (Fig. 13) has been reported as a phytoplasma vector of Weligama coconut leaf wilt disease in Sri Lanka (Kumara et al., 2015). It is a monophagous species breeding only on the inflorescence of mango. However, adults are found on several plants during non-flowering season of mango (Viraktamath, 1989).

Yamatotettix fulvivittatus Matsumura: This species belongs to the tribe Macrostellini of the subfamily Deltocephalinae. This tribe contains large number of phytoplasma vectors in the world. This species occurs in northeast India and its close relative Yamatotettix sexnotatus (Izzard) occurs throughout India on sugarcane (Webb 1986). Y. fulvivittatus is known to transmit sugar cane white leaf phytoplasma and the pathogen is also trans-ovarially transmitted (Hanboonsong, 2006).

Olidiana kirkaldyi (Nielsen): This Coelidiinae leafhopper was earlier misidentified as Jassus indicus (Walker) which was incriminated as the vector of sandal spike phytoplasma (Rangaswamy and Griffith, 1941; Nielsen, 1982) and was described as a new species, Calodia kirkaldyi Nielsen. It is now placed in a genus Olidiana Nielsen. C. kirkaldyi (Figs. 20, 30) is a large species feeding on Calotropis gigantea (L.) W.T. Aiton, Tectona grandis L. f., Morinda tinctoria Roxb., Bambosa sp., Morus alba L., Santalum album L., cotton (Gossypium spp.), horse gram (Macrotyloma uniflorum (Lam.) Verdc.). The nymphs and adults of this species are seen on Dodonaea viscosa Jacq. and Ziziphus oenoplia (L.) Miller suggesting that these may be the breeding hosts (Subba Rao et al., 1988). Its ability to transmit sandal spike phytoplasma needs to be confirmed.

Alebroides nigroscutellatus (Distant): This is a slender cream coloured leafhopper belonging to the second largest and one of the most advanced clade of leafhopper sub-families Typhlocybinae. It was earlier known as Alebroides dravidanus (Ramakrishnan & Menon). This transmits potato purple top roll phytoplasma (Singh and Nagaich, 1977) (Table I). It is widely distributed in India and Singh et al. (1983) report that the phytoplasma caused some adverse effects on the vector. The leafhopper apparently breeds on potato.

Amrasca devastans (Distant): This species (Fig. 21) commonly known as cotton leafhopper [also known as A. biguttula biguttula (Ishida)] is a very polyphagous leafhopper breeding on economically important plants across several families including Malvaceae and Solanaceae. Maramarosch et al. (1970) reported it to transmit little leaf of brinjal which however, need to be confirmed.

Mesargus albomaculatus (Distant): This species earlier known as Moonia albomaculata Distant (Figs. 22, 33) was suspected as a vector of sandal spike (Table 1). However, its ability to transmit the phytoplasma needs to be confirmed. It breeds on several hosts including sandal and the creeper, Dolichandra unguis-cati. (L.) Miers Both nymphs and adults are more
sedomary feeding on shoots and pencil thick stem of these plants.

**Nilaparvata lugens (Stål):** The brown planthopper (Figs. 23-25) belongs to the family Delphacidae of the superfamily Fulgoroidea. It is a common species in rice ecosystem and monophagous breeding only on the species of *Oryza*. As in the case of adult leafhoppers, these also probe on several plants both monocots and dicots. It is known to transmit several viruses of rice plant however, it is suspected to transmit an unknown phytoplasma affecting rice (Table 1). It has a very complex life cycle and exhibits wing polymorphism based on population density and host plant suitability (Wilson and Claridge, 1985).

**Redarator bimaculatus Distant:** This Issid planthopper of the superfamily Fulgoroidea is reported as a vector of sandal spike disease in Kerala (Table 1). *R. bimaculatus* (Figs. 26, 27) is a very distinctive Issid planthopper with prominent pair of facial black spots which sometimes coalesce to form one transverse band on upper part of face. No further confirmation of this has so far been published and hence it needs to be proved that *R. bimaculatus* indeed is also one of the vectors of sandal spike. Information on its host plants and biology is not known.

**Proutista moesta (Westwood):** *P. moesta* (Fig. 34) is a beautifully coloured derbid planthopper belonging to the family Derbidae and superfamily Fulgoroidea. The nymphs usually breed in decaying vegetable matter feeding on fungi and adults are found on palms and also on crops like maize and sorghum often in large congregations. *P. moesta* is considered as the main vector of coconut root (wilt) phytoplasma and also areca nut yellow leaf phytoplasma found in south India (Table 1).

**Halyomorpha halys Stål:** This is a medium sized Heteroptera bug belonging to the family Pentatomidae. This species does not occur in India, but its close relative *Halyomorpha picus* (Fabricius) (Fig. 28) is a common species affecting areca nut, pigeon pea, cotton, pomegranate, cowpea, lablab, tamarind, etc. *H. halys* is an invasive pest in the United States of America and is also known to transmit phytoplasma (Table 1).

**Stephanitis typica (Distant):** This is another suspected Heteroptera bug belonging to the family Tingidae and superfamily Miroidea vectoring phytoplasmas. Both nymphs and adults of *S. typica* (Fig. 29) feed on under surface of leaf and all stages can be found there. They feed on mesophyll and remove cell contents thus the affected leaf shows silvery stippling symptoms which later coalesce and form large grey patches. This species is reported as vector of coconut root (wilt) disease in south India (Table 1). However, this needs to be confirmed.

### The bacterium, Xylella fastidiosa

The bacterium, *Xylella fastidiosa* is a Gram negative plant xylem restricted gamma-Proteobacterium living as endosymbiont in several plants and a few of its clades causing serious diseases in several economically important plants (Janse and Obradovic, 2010). More than 563 plant species in 82 families harbour this bacterium, some asymptomatic and others showing symptoms of leaf scorching and stunting. The entire genome of this bacterium has been sequenced and usually five subspecies are recognised: (i) *Xylella fastidiosa* subsp. *fastidiosa* Wells et al., Pierce’s Disease (PD) and Almond Leaf Scorch (ALS), strains from cultivated grape, alfalfa, almond, and maple; (ii) *X. fastidiosa* subsp. *multiplex* Schaad et al., Phoney Peach Disease (PPD), Plum Leaf Scald (PLS), strains from peach, elm, plum, pigeon grape, sycamore and almond; (iii) *X. fastidiosa* subsp. *pauca* Schaad et al., citrus variegated chlorosis (CVC), strains from citrus and probably those from coffee leaf scorch (CLC); (iv) *X. fastidiosa* subsp. *sandyi*, strains from *Nerium*, Oleander Leaf Scorch (OLS); (v) *X. fastidiosa* subsp. *tashke*, strains from the ornamental tree *Chitalpatash kentensis* T.S. Ellis & Wisura. Pierce’s Disease (PD) of grapevine, Citrus Variegated Chlorosis (CVC), Almond Leaf Scorch (ALS), Oleander Leaf Scorch (OLS), and Olive Quick Decline Syndrome (OQDS) are among the most economically significant *X. fastidiosa* related diseases in the world. Leaf scorch symptoms and ultimately plant death are thought to be the outcome of bacterial growth and clogging up of xylem vessels (Hopkins, 1989).

This bacterium is transmitted by the xylem sap feeding Hemiptera that include Cicadidae (Cicadoidea), Cercopidae, Macherotidae, Aphrophoridae (Cercopoidea) and Cicadellidae (mainly the species of the subfamily Cicadellinae). As a vector-borne pathogen, plant-to-plant spread of *X. fastidiosa* is directly related to the presence, abundance, and behaviour of insect vectors in relation to infected and healthy plants. The relationship of the bacterium with its vector is very unique. The bacterium is transmitted in non-circulative but propagative manner. Both nymphs and adults can transmit the bacterium but the nymphs...
lose their infectivity after a moult (non-transstadial). The bacterium is also not trans-ovarially transmitted. The vectors have a very short AAP lasting for a few hours, however, more the AAP better is the transmission and there is no appreciable latent period (Redak et al., 2004). After acquisition, the bacterium is retained on the cuticular lining of the insect foregut, likely in the part of the pre-cibarium proximal to the cibarium (Almeida and Purcell, 2006). It also multiplies in these areas and forms biofilm. A number of species of Cicadellinae and Cercopoidea can transmit the bacterium but with varying transmission efficiency (Almeida, 2016). As far as PD in California is concerned, Graphocephala atropunctata (Signoret) is the most efficient transmitter. However, the disease was almost under control after the first outbreak during 1930s. It was shown that the occurrence of PD was much higher in the grapevines that are bordering riparian areas from where the leafhoppers could arrive during spring and infect the vines. However, secondary spread was not very high as the leafhopper G. atropunctata and Draeculacephala minerva Ball and other native cicadelline vectors feed on new shoots and fresh leaves and the bacterium did not have enough time to invade woody tissue and these parts are pruned during winter pruning thus limiting its spread to woody tissues. However, with the introduction of Glassy-Winged Sharp Sooter (GWSS) Homalodisca vitripennis (Germar) [earlier known as H. coagulata (Say)] in California changed the entire scenario and there was severe epidemic of the disease threatening the entire grape industry. H. vitripennis is considered the most important vector despite its relatively inefficient transmission rates (Almeida and Purcell, 2003). Its high mobility, extreme polyphagy, lack of biological control organisms, high densities in preferred habitats from which they disperse, and wide geographical distribution due to transport of commercial nursery products offset its poor transmission ability (Redak et al., 2004). GWSS feeds both on young shoots and older stem and is also capable of transmitting the bacterium to dormant canes. When the bacterium is in the woody part of the vine it is protected from the low winter temperature and by the spring it can infect the plant. Thus GWSS plays an important role in secondary spread of the bacterium leading to the epidemics. This also led to considerable research on biology and ecology of the vectors, the pathogen and its epidemiology. Various factors influence vector transmission of X. fastidiosa, including the distribution and density of bacterial populations in host plants, insect host range and plant preference, season of inoculation, and climatic conditions. The ecology of vectors can affect epidemics, as demonstrated by the large increase in PD of grapevine incidence in California after the introduction of GWSS (Almeida et al., 2005).

The first confirmed report of X. fastidiosa in the Old World was from Taiwan during 1980s when pear leaf scorch disease was reported (Leu and Su, 1993) and later PD was reported in grapevines in Taiwan (Su et al., 2013). Search for the vector showed that Kolla paulula (Walker) and Bothrogonia ferruginea (Fabricius) transmitted PD with transmission rates of 13.3 and 6.7%, respectively, in grapevine (Tuan et al., 2016). The aphrophorid, Poophilus costalis (Walker) was also suspected as a vector of PD (Su et al., 2011). These species of insects are also present in India. X. fastidiosa has been reported in several Asian countries however, as stated by EFSA (2015), some of the detections appear to be unconfirmed namely, that from India (Jindal and Sharma, 1987) and Turkey (Guldur et al., 2005).

In Europe, the first confirmed report of X. fastidiosa was from France in coffee plants in containment. However, its field establishment was that from Italy (Saponari et al., 2013) affecting olive trees (Olea europaea L.) growing on the west coast of Salento Peninsula, resulting in the decline of the trees because of an unknown disease subsequently named Olive Quick Decline Syndrome (OQDS) (Martelli et al., 2016). The report of the bacterium from Germany is now reported as eradicated. Europe does not have many species of Cicadellinae. One very widely distributed species, Cicadella viridis (Linnaeus) (in the Palaearctic region) is considered as the vector. However, Cercopoidea and cicadas, which are considered to be marginally important vectors in other continents, are thought to play a key role in X. fastidiosa epidemiology in Europe. So far, the role of cicadas in transmitting X. fastidiosa has not been well established and considering their short adult life-cycle they may be of very minor importance as vectors. Among the Cercopoidea, the polyphagous and wide spread meadow spittlebug, Philaenus spumarius (Linnaeus) (family Aphrophoridae) is considered the most important vector responsible for the spread of the bacterium in olive orchards (Cornara et al., 2017).

Auchenorrhynch that could serve as vectors of Xylella fastidiosa in India

X. fastidiosa does not have strong vector specificity and a number of xylem feeding Hemiptera can transmit them with varying degree of efficiency. As there are
no confirmed plant diseases caused by this bacterium reported from India, here only those genera which are more common and can probably serve as vectors are mentioned.

Species of Cicadellinae and Signoretiinae:
As mentioned earlier, the leafhopper subfamilies Cicadellinae and Signoretiinae include those leafhoppers which feed on xylem sap and have greatly enlarged faces to accommodate strong muscles required to pump the xylem sap which is under negative pressure.

The following common species of Cicadellinae may serve as vectors of *Xylella*. *Anagonalia melichari* (Distant) (Fig. 37), *Anatkina helena* (Distant) (Fig. 38), *Atkinsoniella opponens* (Walker) (Fig. 39), *Bothrogonia albidicans* (Walker) (Fig. 40), *Bothrogonia sclerotica* Young (Fig. 41), *Bothrogonia ferruginea* (Fabricius), *Cofana lineata* (Distant) (Fig. 42), *Cofana unimaculata* (Signoret) (Fig. 43), *Cofana spectra* (Distant) (Fig. 44), *Kolla ceylonica* (Melichar) (Fig. 45), *Kolla paulula* (Walker) (Fig. 46) and the species of the subfamily Signoretiinae, *Signoretia* sp. (Fig. 47). The adults of these leafhoppers are very common on different plant species either on the new foliage (*K. ceylonica*, *K. paulula* and *Signoretia* sp.) or on leaves and shoots of different species of plants in moist

![Leafhoppers that probably act as vectors of *Xylella fastidiosa* if it gets accidentally introduced into India](image)
forests. Both nymphs and adults can act as vectors of Xylella. K. paulua and B. ferruginea have already been demonstrated as vectors of X. fastidiosa causing Pierce’s Disease of grapevine and Pear leaf scorch disease in Taiwan (Tuan et al., 2016).

**Species of Cercopoidea:** The species of Cercopoidea belong to three families namely Aphrophoridae, Cercopidae and Macherotidae. Of these the species of the first two families may be important as vectors of Xylella. Both nymphs and adults are xylem feeders. The nymphs are in froth (spittle) and are sedentary and my not play any role in transmission of Xylella. Only the adults are able to transmit the pathogen with varying degrees of efficiency. Some of the species which can act as vectors of Xylella in the Indian subcontinent are as follows: Aphrophoridae: Aphrophora sp. (Fig. 48), Clovia puncta (Walker) (Fig. 49), Clovia lineaticollis (Motschulsky) (Fig. 50), Poophilus costalis (Walker) (Fig. 51) and Ptylinellus praefectus (Distant) (Fig. 52); Cercopidae: Callitetix versicolor (Fabricius) (Fig. 53), Eoscarta sp. (Fig. 54) and species of Cosmoscarta (Fig. 55). Among these, species of Aphrophora, Poophilus, Ptylinellus, Callitetix, Eoscarta and Cosmoscarta are found on woody trees and shrubs in addition to grasses. C. lineaticollis is found breeding on jack fruit saplings and trees and C. puncta breeds extensively on grasses and the adults feed both on grasses and herbs and shrubs. The Indian fauna of Cercopoidea is in need of revision as it is difficult to identify the species based on century old descriptions given by Distant (1908, 1916).

**Search for the unknown vector(s) of phytoplasmas**

The knowledge of the insect vector is crucial for well-timed and efficient control strategies of phytoplasma diseases and to avoid further spread of the pathogen (Alma and Tedeschi, 2011). In the case
of new phytoplasma diseases, the vectors are always unknown and intensive studies are required to identify the insects. Similarly, when a new outbreak occurs in new geographical areas, the already known vectors may not have the same role and the presence of other possible vectors need to be explored.

Insect sampling for vector search

Different sampling techniques need to be employed not only on the crop plant but also on associated plants including weeds and shrubs in the diseased area. Sampling also has to be done during different seasons of the year as the life cycle of the insect vectors may differ. Once the putative vector is identified the knowledge of its wild host plants becomes of utmost importance to understand its ecology and behaviour and the epidemiology of the disease.

When nothing is known, our aim should be to sample large number of insect fauna from the diseased area. A number of insect collection methods that are available could be adopted for collecting vector Auchenorrhyncha. However, till the time the vector groups are narrowed down, general collection methods in the area of prevalence of the phytoplasma disease could be used. These could be sweep net collecting on vegetation, light traps, malaise traps, yellow pan traps, beating tray and yellow sticky traps. These would yield large number of insect samples. Details regarding how to set up and use these methods could be found in Triplehorn and Johnson (2005, and the references therein). The sweep net and beating tray will yield live insects, whereas the others are likely to yield only dead insects, unless they are manually attended as in the case of light traps. Some collection equipment like Malaise trap, light traps are useful as they act as random samplers irrespective of the host plants of the insects. Information on place of collection, methods used, date, name of the collector and the host plants if available need to be carefully recorded and documented. However, the material collected need to be sorted out and the Auchenorrhyncha fauna need to be identified and could also be used for testing whether any of them contain phytoplasmas of interest. Sticky traps can be used for trapping insects associated with different plant species and need to be serviced more frequently to get better quality dead insects for further study and identification. To collect live insects for developing cultures and other laboratory studies hand picking through aspirator, sweep nets and beating trays would be most useful. In addition to live insects, these methods can give us information on host plants and natural enemies of the insects collected. The vacuum insect collector (D-VAC) is a very useful instrument to sample live insects from dense vegetation, thorny plants and very low vegetation close to ground to collect surface dwelling vectors which may be missed otherwise.

Ones the groups of Auchenorrhyncha that harbour the phytoplasma of interest are narrowed down either to genus or to species, specific methods could be devised to selectively collect different stages of these based either on the literature or by the advice of the experts or field observations and also their host plants and preferred habitats, ecological conditions, seasonal incidence behaviour etc. could be studied.

Handling collected insects

Dead insect samples thus collected need to be sorted, labelled (locality label including data on place, date of collection, host plants if any, method of collection and collectors name) individually after mounting them appropriately depending on their size (either pinning them directly or mounting them on a triangular paper point or preserving in 75-80% ethanol, labels in alcohol have to be either printed or written on soft paper with pencil) and identified. Triplehorn and Johnson (2005) may be consulted for further details.

Cooperation of well-trained taxonomists need to be sought and can be built up right at the beginning of the project proposals for studies on vector borne plant pathogenic diseases. The technicians involved in the project may be trained in preliminary identification of the species of Auchenorrhyncha and Sternorrhyncha groups of phytoplasma vectors. They also need to be trained in the preparation of wing and male genitalia and should be conversant with the terminologies used. Identification of these groups is mainly based on the detailed study of the male genitalia and any deficiency in that would affect accurate identification. They should also be trained in the identification of immature stages and sexes. It should also be noted that the vectors involved could be un-described taxa and help of a specialist in the group may be needed to address this issue. It is also necessary to preserve the voucher specimens used in the investigation in a recognised insect collection. It is also advised to clearly mark the proven vectors and material used in molecular identification etc. for future reference and verification if needed.

When live samples are collected, they need to be placed in clean vials along with shoots or leaves of their host plant and brought to the laboratory and maintained
on their respective host plants for further studies in the laboratory. Here also species need to be sorted out and reared on their respective host plants.

Transmission studies

Experimental transmission studies are essential to ascertain the vector ability of putative vector species. For this the test insect could be naturally infected or experimentally infected (Alma and Tedeschi, 2011).

Experiments with naturally infected insects

1. Healthy insects (usually laboratory reared) are caged on infected plants for Acquisition Access Period (AAP). The AAP may be for 24, 48, 72 hours or even longer if needed.
2. After AAP the insects are removed to suitable healthy plants to complete their Latency Period (LP). The LP may be for 8, 10, 15, 20, 30 days or even more if required.
3. After LP the insects are transferred to healthy plants for Inoculation Access Period (IAC). This may last for a few days to several weeks. The number of insects used, stage of insect used and also the sexes used may be varied to test their relative efficiency.
4. After IAC, the insects are removed and tested for the presence of phytoplasma using molecular techniques. The plants are maintained in insect proof cages for symptom development which may take a few to several months to appear. The plants may also be tested for the presence of the phytoplasma using molecular techniques. In woody plants, the symptoms may appear very late even after one year or more and phytoplasma titre could be very low, thus not detectable for months.

Field collected insects (putative vector species) could also be used for transmission studies in preliminary experimental stages, if there is difficulty in establishing laboratory culture of putative species.

All these studies need to be done in insect proof cages in greenhouse condition so that the researchers know that the healthy plants are really healthy and the insects specimens used are also healthy. These experiments need to be carefully planned well in advance so that the required number of healthy plants and healthy insects to be tested is always available for the experimentation. It is also necessary to have a good infected source plant with accurately identified phytoplasma.

Tests with experimentally infected insects

Transmission to artificial feeding medium is a good and practical alternative to test the inoculative ability of the candidate insect vector (Tanne et al., 2000; Bressan et al., 2006; Pinzonti et al., 2008). In this procedure the insects after LP (see previous section) are allowed to feed on artificial feeding medium such as sucrose or sucrose plus sorbitol with buffer solution. After IAP the insects are removed and tested for the presence of phytoplasma. The feeding medium is removed and frozen and tested for the presence of the released phytoplasma using molecular procedures.

Vector search on fast track

Search for the insect vectors of a vector transmitted plant pathogens take considerable time and energy. However, with the advancement in the molecular biology, tissue culture and rearing techniques in Hemiptera, it is possible to reduce the quantum of Hemiptera to be tested and the time required to find out putative vectors those can be subjected to more rigorous tests for their role in transmission of the pathogen in nature.

Some of the pre-requisites for fast tracking the search are the following:

(a) Availability of tissue culture techniques for production of large number of young susceptible host plants of the pathogen especially in the case of perennial plants like coconut, arecanut or sandal. If such a technique is not available then, identification of annual plant species if any which can act as laboratory hosts for maintenance and transmission of the pathogen.

(b) Availability of good source plant of the pathogen under study. Ensure that it is not a dead-end host. The pathogen needs to be periodically transferred to young plants so that the pathogen is in active multiplication state for further experimentation.

(c) Developing molecular identification techniques for the pathogen both in the plant and in the vectors.

Procedure

1. Collect live auchenorrhynchan insects from both diseased plants, healthy plants and also from surrounding vegetation and maintain them separately. Pay particular attention to specimens collected on diseased plants.
2. Sort them into species. Allow 5-10 specimens of each species separately to feed on source plant
Infected with the pathogen under investigation and maintained in the laboratory for 24-48 hours (AAP) and transfer them to healthy plants for completing their latent period.

3. After 8-10 days (or more days) of LP, allow a batch of 5 specimens of each species to feed on 3-5 ml of buffered sugar solution for about 24-48 h IAP. Test the fed solution for the presence of pathogen using molecular technique and also test the insects for the same. Those insect species that released the pathogen in the sugar solution when feeding are the vectors. These insects can further be tested rigorously for transmission using the susceptible young host plants.

4. Those insect species which tested positive for the pathogen but the sugar solution on which they fed tested negative may not be the vectors of the pathogen.

5. One can also test the collected specimens for the presence of pathogen individually or species-wise and narrow down the species that carry the pathogen and then use the steps 2 and 3 mentioned above.

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In search of insect vectors of phytoplasmas with a note on vectors of *Xylella fastidiosa*

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