Indian Journal of Entomology 85(4): 1105-1114 (2023) DoI. No.: 10.55446/IJE.2022.455

EMERGING PHYTOPLASMA DISEASES IN FORESTRY AND THE ROLE OF DETERMINING THE INSECT VECTORS FOR THE MANAGEMENT OF THE DISEASES#

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ABSTRACT

Pathosystem associated with phytoplasmas in forest plants is an emerging threat to forest productivity and biodiversity. Diseases caused by or associated with phytoplasmas occur in hundreds of commercial and native plants, causing minor to extensive damage. Phytoplasmas are wall-less, phloem-limited, insect-transmitted, plant pathogenic bacteria that are transmitted by phloem-sap feeding insects of the order Hemiptera under the families such as Cicadellidae, Delphacidae, Cixiidae, Derbidae, Psyllidae and rarely by Pentatomidae and Tingidae. Disease transmission occurs very quickly, often plants become infected before adopting any management strategies. The single most effective management practice of managing the vector in agricultural/horticultural crop is to cover plants with insect exclusion netting but this is not feasible and practical in the case of forest trees. Because of these limitations, researchers are shifting to manipulation genetics in host plants to affect vector populations and transmission of pathogen but big lacunae exists in our knowledge on the vectors of phytoplasma diseases in Indian forestry. We review the literature addressing the phytoplasma diseases in forest species, the lacunae existing in the insect vectors and the need for determining the vectors for possible management of phytoplasma diseases in forest trees.

Key words: Hemipteran vectors, forest species, symptoms, management

Yellow diseases of trees and shrubs are among the most important diseases in forests as they cause very extensive and serious damage in perennial crops, particularly in woody plants. Such plants if infected once, the pathogen is carried throughout the life of the host plants. More than 60 forest species, trees, shrubs and herbs have been reported infected by the pathogens causing yellows and witches' broom (Nayar, 1988b). In early years, the causative organisms are termed as Mycoplasma-Like Organisms (MLOs) but presently they are classified as Phytoplasmas and are a large group of obligate, endophytic, cell wall-less bacterial parasites classified within the class Mollicutes (Wei et al., 2007). Pathosystem associated with phytoplasmas in forest trees is an emerging threat to forest productivity and biodiversity. They are fastidious prokaryotes that can survive and multiply only in hypotonic habitats such as plant phloem or insect haemolymph and they are strictly host dependent. They are known to be pathogenic to more than 300 plant species (Bertaccini et al., 2014). In India presently more than 172 plant species are reported to be the host of ten different groups of phytoplasmas associated with plants including vegetables, fruits, trees, ornamentals, sugarcane, palms, oil crops and weeds are increasing at an alarming rate (Rao, 2021). Phytoplasmas are mainly transmitted by phloem- sucking leafhopper species, primarily belonging to the three families, Cicadellidae, Cixiidae, and Psyllidae (Weintraub and Beanland, 2006). The distribution of phytoplasma diseases in different geographical areas and their impact depends on the host range of the phytoplasma as well as the feeding behaviour of the insect vectors. Phytoplasmas are complex syndrome with symptoms such as phyllody, forming sterile deformed flowers, proliferating auxiliary shoots, virescence and stunting in several hundreds of plant species (Lee et al., 2000). The major phytoplasma diseases causing severe economic losses in the country are sesame phyllody, brinjal little leaf, sugarcane grassy shoot, sandal spike, coconut root wilt and areca nut yellow (Rao et al., 2017). So far ten 16Sr groups of phytoplasmas have been identified in different plant species. The biology, ecology, vector interaction, and epidemiology of crop diseases caused by phytoplasmas, are reviewed extensively in recent years by Christensen et al. (2005), Weintraub and Beanland (2006),

#Paper presented in the discussion meeting on "Challenges in the Management of Phytoplasma Diseases" held at Institute of Wood Science and Technology (IWST), Bangalore, on 5th February 2021 (Editors: R. Sundararaj and N. K. Krishna Kumar)

Bertaccini (2007), Rao et al. (2017), Marcone (2021) and Rao (2021). For many phytoplasma diseases in India that are known more than a century such as sandal spike disease, coconut root (wilt) and areca nut yellow leaf diseases, information on their confirmed insect vectors are not known, only putative vectors are known so far. Though insects remain the most significant vectors of these organisms, unfortunately, information on them as vectors of phytopathogens in universal and phytoplasmas in particular is meager. The significance of phytoplasma diseases in important forest species and necessitate to investigate their insect vectors are discussed in detail in this manuscript.

Phytoplasma diseases in forest plant species

Nayar (1988a; 1988b) reported phytoplasmas in more than 60 forestry plant species, including 19 economically important species (Table 1). Among the listed ones, four species belongs to the family Fabaceae, two each in the family Arecaceae, Poaceae, Rhamnaceae and Santalaceae. Remaining families viz., Acanthaceae, Lamiaceae, Meliaceae, Myrtaceae, Sapindaceae and Rubiaceae are represented by each one species.

Sandal spike disease (SSD) of Indian sandalwood

Indian sandalwood (*Santalum album* Linn.) is a hemiparasitic, perennial tree belonging to the family Santalaceae. It possesses a highly valuable wood, known for its scented oil and carving and which is acknowledged as "*Royal Tree*" in Indian continent is one of the most economically important tree species (Sundararaj, 2008). In India, sandal is found mainly in the Deccan Plateau and its extension and in small numbers in almost all parts except the Himalayas. Large natural stand of sandal occurs in Karnataka (5,245 km^2) followed by Tamil Nadu (3,040 km^2) accounting for nearly 90% of sandal in India (Venkatesan, 1981). The price of Indian sandalwood and its oil have risen significantly in the past decade mainly due to depletion in production annually at the rate of 20% since 1995 (Ananthapadmanabha, 2000). India's production during 1930s to 1950s was around 4000 tons of heartwood per year which had decreased to an average of 300 tons per year. Correspondingly the wood price from 1996 to 2012 has increased in arithmetic progression reached Rs. 60,00,000 per ton in 2013. It has impacted the oil export as the maximum quantity of 27,930 Kg sandalwood oil exported in the year 1997-98 has reduced to 2,330 kg in 2019-20 (Export-Import Data Bank V.7.1 Trade Stat, 2021).

The decline in sandalwood production is mainly due to the depletion of sandalwood trees in its natural habitats of forest. The major reasons for this reduction is illicit felling and smuggling and the other reasons being the impact of pests and disease (Rao et al., 1999). They cause serious disease of sandalwood, Sandal Spike Disease (SSD) known from 1850 in Karnataka state, caused havoc in the production of sandalwood (Nayar, 1988a) and are one of the major causes for the

Table 1. Phytoplasma diseases associated with important forestry plant species of India (From Nayar, 1988b)

Sl. No.	Tree/Shrub species	Family
1	*Barleria mysorensis* Roth.	Acanthaceae
2	*Areca catechu* L.	Arecaceae
3	*Phoenix sylvestris* (L.) Roxb.	Arecaceae
4	*Acacia catechu* Wild	Fabaceae
5	*Acacia intsia* Willd	Fabaceae
6	*Dichrostachys cinerea* W.et.A.	Fabaceae
7	*Pongamia pinnata* (L.) Pierre	Fabaceae
8	*Gmelina* sp.	Lamiaceae
9	*Cedrela toona* M. Roem	Meliaceae
10	*Eucalyptus* spp.	Myrtaceae
11	*Ochlandra travancorensis* (Bedd.) Benth. ex Gamble	Poaceae
12	*Dendrocalamus strictus* (Roxb.)	Poaceae
13	*Scutia indica* Bronga	Rhamnaceae
14	*Ziziphus oenoplia* (L.)	Rhamnaceae
15	*Randia candolleana* W.et A.	Rubiaceae
16	*Dodonea viscose* (L.) Jacq.	Sapindaceae
17	*Ozyris* sp.	Santalaceae
18	*Santalum album* L.	Santalaceae

decline in sandalwood production as the quantum of trees extracted due to SSD is enormous. Over a million of sandalwood trees had been removed in Coorg and Mysore (Karnataka) during 1903-1916 (Mc Carthy, 1899; Hodgson, 1918; Coleman, 1923; Rangaswami and Sreenivasya, 1934) and 98,734 trees during 1917-1925 in Salem (Tamil Nadu) alone due to SSD (John, 1957). In Karnataka, the growing stock has been condensed to 25% of its initial level in the last two decades of 20th century (Swaminathan et al., 1998). The disease is not known in Timor and does not affect other species of *Santalum* (Fox et al., 1994). All these mass extractions due to SSD in natural habitats pushed the sandalwood to be first classified as vulnerable by the International Union for Conservation of Nature in the year 1998. Even in the global scenario, sandalwood has developed into a commercial timber crop over the past 10 to 15 years, with substantial sandalwood plantations established in India, China and Australia, and more modest plantings being established in Fiji, Vanuatu, Hawaii and Indonesia. In this context, it is key note that the SSD is so far reported only from India and the research on SSD in India is more than 50 years old, Realizing the sharp decline in the sandalwood population in the states and having realized that they cannot be the custodian of sandalwood any more, Karnataka and Tamil Nadu forest departments have amended the Sandalwood act in 2001 and 2002, respectively and made growers themselves as the owners of the sandalwood as per the Amended Acts. The purpose of the amendment is to encourage farmers and corporate bodies to grow sandalwood plantations to meet the growing demand (Gowda et al., 2008). This is encouraging the farming community and private entrepreneurs to cultivate sandalwood in agro-forestry, farm forestry and varied agri-silvi-horticultural and mixed plantation systems as per their choice. Its hemiparasitic nature makes it suitable for tree species in any agro-forestry conditions (Sundararaj et al., 2019).

Causative agents of sandal spike disease

SSD is the most serious disease of *S. album* which is characterized by extreme reduction in the size of leaves and internodes accompanied by stiffening of the leaves. In advanced stage, owing to the progressive reduction in leaf size and internodes, the whole shoot looks like a 'spike inflorescence'. Though it was known from 1850 in Karnataka, it was first reported in Coorg, South India (Karnataka) in the year 1899 (Mc Carthy, 1899). It was initially considered as viral disease and then as the disease caused by Mycoplasma Like Organisms (MLO) (Parthasarathi et al., 1966; Hull et al., 1969), and presently as Phytoplasma disease. At present, natural populations of Sandalwood are mainly available in Marayur in Kerala and some patches of reserve forests of Karnataka. Recently it is confirmed that in these natural habitats the trees are heavily infected with SSD associated with *Candidatus* Phytoplasma asteris (Kirdat et al., 2019) and 1 to 5% trees are dying annually (Figs. 1-4). The evidence suggests that only 18 months is enough to cause the death of a sandalwood tree by SSD and it kills even very young naturally regenerating trees before reaching the age of heartwood formation. Disease incidences reaching up to 33 and 55% were recorded in southern Karnataka (Murali et al., 2019).

Rising threat of sandal spike disease

The situation in Marayur sandalwood reserve which had a density of about 1000 sandal trees per hectare (Nayar, 1988b) was considered relatively free from SSD till 1980 (Prasad, 2011). It recorded 21.4% density of sandalwood in 2005-06 (Sundararaj and Sharma, 2010) which is now reduced to 1.92% mainly due to the death of trees by SSD and consequent extraction on annual basis. Similar situation of dying of naturally regenerating trees happens in the natural habitats of sandalwood in Karnataka and the sandalwood plantations raised by the Karnataka Forest Department. The potential phase of spreading of the infection is primarily due to the fact of restriction on green felling in forests, which allow the infected tree to serve as inoculums till it dies and spread the phytoplasma pathogen to the maximum extent to the healthy trees by the potential vectors.

Phytoplasma diseases in bamboos

Bamboos are interesting plants that profusely grow in the tropical and subtropical zones and they form the most important non-woody species (Naithani, 1993). They represent the world's best natural and renewable resources with exceptionally diverse group of species (Seethalakshmi et al., 2008). There are more than 1250 species of bamboo under 75 genera worldwide, which are unevenly distributed in various parts of the humid tropical, sub-tropical and temperate regions of the earth (Subramaniam, 1998). A total of approximately 148 species in 29 genera of bamboos currently occur in India (Sharma and Nirmala, 2015). Bamboos form an important group of tropical woody monocotyledons that have been traditionally used by people in Asia for a wide variety of purposes and they have been regarded as the major resource that meets the multiple requirements of common people and also a poverty alleviator (IFAD, 2014). It is estimated that about 8.6 million people depend on bamboo for their livelihood in India (FSI, 2019). There is a growing trend of using bamboo in

Figs. 1-2. Sandalwood trees in various stages SSD in Marayur sandalwood reserves

Fig. 3- 4. Drying and dyeing of sandalwood trees due to SSD at Marayur sandalwood reserves

rural and urban areas of the country as a widespread, renewable, productive, versatile, low cost or no-cost, easily accessed, environment-enhancing resource. The importance of bamboo as a resource for livelihood development and alleviation of both environment and social problems is increasing (Singh et al., 2017). Many diseases such as leaf spot disease, leaf blight, and seedling wilt in nurseries, bamboo mosaic disease, witches' broom, little leaf disease, and emerging culm rot affect the bamboos in plantations (Harsh et al., 2005). Among these diseases, the little leaf disease is caused by phytoplasma and it is known to infect *Dendrocalamus strictus* (Yadav et al., 2015), *Bambusa bambos* (L.) Voss, *D. stocksii* (Munro.) and *D. asper* (Schult.) Baker (Mondal et al., 2019). The infected bamboos show typical disease symptoms of witches' broom with severe proliferative branching at nodal regions and reduction in the size of the leaf. The leaves dry prematurely and look burnt in appearance. The affected culms bend due to higher weight and then dry (Figs.5-7). It attracts the infestations of termites besides such dried culms may catch fire. *Ca* P. aurantifolia was known to cause the symptom of witches' broom disease in bamboos; however, so far no information is available on its insect vectors.

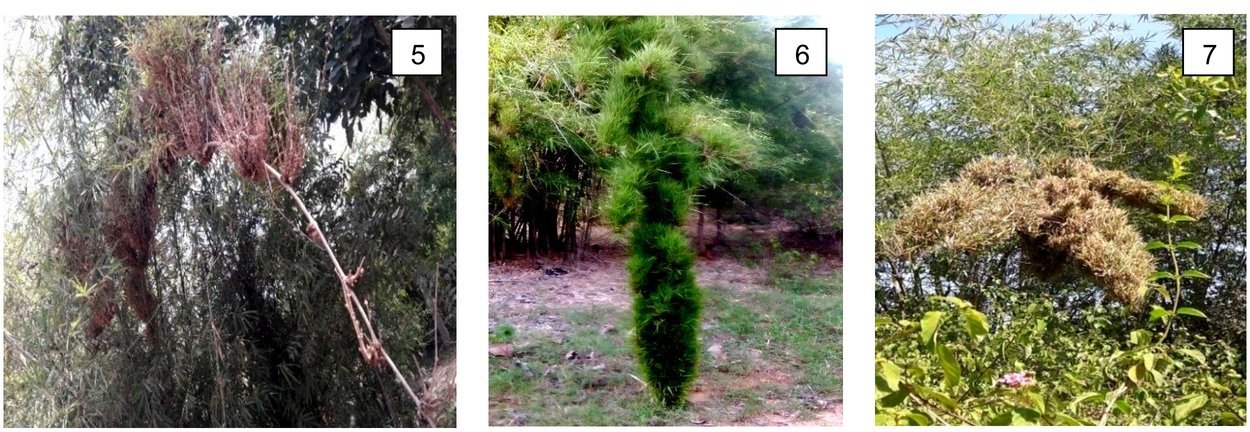
Phytoplasma diseases in other forestry tree species

Phytoplasma disease is also recorded in several Eucalyptus species. This is also called as Eucalyptus Little-Leaf (ELL). It was first observed in India in the year 1971 and was thought to be caused by a virus (Sastry et al., 1971). The disease was found to be induced by *Frankliniella occidentalis* (Pergande). Based on electron microscope observations of diseased eucalyptus trees, it was later proved as phytoplasma infection (Marcone, 2015). The symptoms described from various geographical regions and different *Eucalyptus* spp. are similar. They include small leaves, pale narrow lamina (little leaf), yellowing, and proliferation from axillary buds that confers a broom-like appearance and wilting of foliage. Diseased trees or their symptomatic parts are stunted with die-back symptoms, do not set fruits, and severely affected trees die. A 16SrV phytoplasma was identified in ELL-affected trees in Italy (Marcone et al., 1996).

Recent studies of SSD in Marayur sandalwood reserves in Kerala State exposed the presence of phytoplasma diseases in many trees like ficus (*Ficus* sp.) (Fig. 8), wild ber (*Zizyphus oenoplia*) (Fig. 9) and wild amla (*Emblica officinalis*) (Fig. 10). Similarly cutch tree (*Acacia catechu*) (Fig. 11) was found associated with phytoplasma symptoms in wesern parts of India. Phytoplasmas are harboured by weeds which may act as alternative natural hosts facilitating their spread to economically important plants and thereby increasing economic losses. About 43 weed species are also reported to be the hosts of phytoplasmas (Rao, 2021).

Management of phytoplasma diseases

Among the forestry plant species reported, utmost importance to be given to developing protocols for effective management of SSD as sandalwood is on the vulnerable list of the International Union for Conservation of Nature. No effective control measure of spike disease has so far been evolved. However, attempts have been made in sandalwood plantations on the following lines, where at the early stage of incidence spread of the disease may be lessened to some extent by eliminating the source of infection. Kristensen (1960) in his report, submitted to the Government of India for the control of SSD has made certain proposals under 3 headings viz.,



Figs. 5-7. Bamboos showing different symptoms of Witches' broom disease

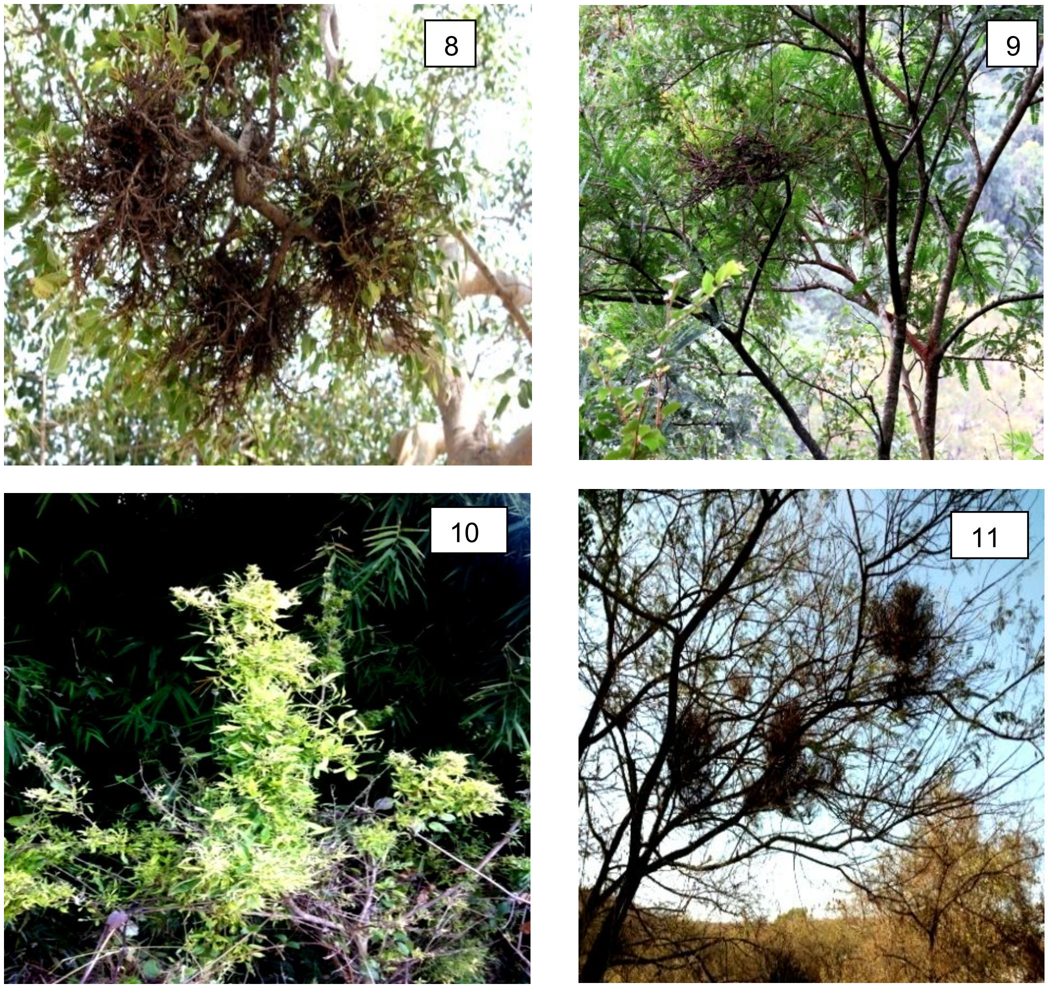


Fig. 8-11. Symptoms of phytoplasma diseases in forest plants. 8. *Ficus* sp.; 9. *Zizyphus oenoplia;* 10. *Emblica officinalis;* 11. *Acacia catechu.*

(i) Diagnostic work: Indexing of various test plants by various transmission methods, and serological work, (The main idea is to detect virus – infected plants and remove the source of infection as early as possible). Accurate diagnosis is necessary for any successful disease control. However, diagnosis of diseases caused by plant molecule has often been one of the difficult aspects. This is primarily due to the lack of methodologies for detecting the pathogens in the field or in quarantine plants (Davis and Lee, 1988). Detection and identification of phytoplasma using molecular techniques like Polymerase Chain Reaction (PCR) has become popular because of the high sensitivity of the test. PCR is preferred in situations where the concentration of phytoplasma is very low.

(ii) Transmission studies: Insect transmission studies to help in the identification of the insect vectors of spike disease. Identification of phytoplasma vectors requires the proof of successful transmission of the pathogens since non-vector species are able to acquire detectable quantities of phytoplasma from infected plants. However, transmission experiments on plants are time consuming and laborious. An alternative technique was developed recently to determine the inoculative ability of potential vectors. (Tanne et al., 2001).

(iii) Control measures: This includes removal of sources of infection, protection against possible vectors, selection and testing of apparently resistant mother-trees of sandalwood. Among these recommendations it is demoralizing that overshadowing all other recommendations only identification of infected plants and their extraction was done because of the financial returns. Considering the impact of SSD in the past, it is imperative to protect the sandalwood populations in their natural habitats from SSD and contain its spread in the farmer's field before it causes colossal damage.

Unlike other crops, the loss of a single tree is crucial for the sandalwood growers as every plant needs to be survived for better monetary returns. This will also guarantee the regaining of the lost glory of the Royal tree of India.

The other forestry important plant species affected by phytoplasmas are Bamboos which are of vital importance from ecological, commercial, and socio-economic points of view. They have more than 1500 documented uses and are widely used for paper and rayon manufacture, construction, architecture, engineering, handicraft, food and medicine. Bamboo forest constitutes about 13% of the total forest area of our country. The bamboos being C-4 plants absorb more carbon-dioxide from the atmosphere and give out oxygen at a faster rate and are better candidates for mitigating climate change. Roots are highly capable of VAM infection, and consequently improve soil conditions, and leaves absorb metals like Fe and Zn. In different tropical forests of the country, bamboos are found to occur widely which apart from being a source of livelihood for forest-dependent population and industry it plays an ecological role in sustainable forest management. Hence, protection of bamboo from phytoplasma diseases requires greater attention.

Importance of vector management for the control of phytoplasma diseases

Phytoplasma-induced diseases in agricultural/horticultural crops are controlled by proper management of insect vectors, removal of infected plants from the field, and by application of plant growth-promoting microbes that not only enhance nutrient levels but also provide protection against microbial and insect vectors (Trivedi et al., 2016). However, so far no potential vector of SSD has been confirmed by applying molecular tests and phytoplasma transmission assays though many insect vectors are suspected to be the transmitters of SSD (Table 2). Unconfirmed transmission of the spike disease by many insects was reported but the validity of the transmission appears doubtful. The disease is known to be transmitted from diseased sandal trees to healthy ones by bud, bark and twig grafting between stems of healthy plants. The idea that vectors transmitted the disease causing agent in nature occurred to early workers when they temporarily prevented the diseases spread by clearing a belt of sandal and *Zizyphus oenoplea* (Coleman, 1917) or when they observed infection in trap plants (Sreenivasaya and Rangaswami, 1934). Prompted by this success, researchers conducted elaborate studies of insect fauna that inhabited sandalwood between 1933 and 1937, and more than 200 species of probable vectors were tested for their ability to transmit spike disease. Hence, studies on vectors to provide conclusive explanation on phytoplasma-vector relationships, under different environmental and farming conditions were inconclusive. Similarly effective management strategies for phytoplasma epidemics are also lacking. Considerable progress has been made in the detection of vectors of phytoplasma diseases of trees outside India (Table 3), such progress is yet to be achieved in the context of Indian forests. Until recently, management of plant diseases caused by phytoplasma was focused on controlling the vector by insecticides, as it is feasible in agricultural/horticultural crops though it is costly, and also not ecologically-sound or economically-sustainable. However, it is practically impossible in forest environment. Reducing the alternative host plants

Table 2. Putative insect vectors of sandal spike disease in *S. album*

Scientific Name	Order: Family	Reference
Coelidia indica (Walker)	Hemiptera: Cicadellidae	Sen-Sarma (1980)
Olidiana kirkaldyi (Nielson)	Hemiptera: Cicadellidae	Sen-Sarma (1980)
Moonia albimaculata Dist.	Hemiptera: Cicadellidae	Sen-Sarma (1980)
Nephotettix virescens (Dist.)	Hemiptera: Cicadellidae	Sivaramakrishan and Sen-Sarma (1977)
Redarator bimaculatus Distant	Hemiptera: Issidae	Balasundaran and Muralidharan (2004)
Jassus indicus (Walker)	Hemiptera: Cicadellidae	Rangaswami and Griffith (1941)
Indomias cretaceous (Faust)	Coleoptera: Cucurlionidae	Hart and Rengaswamy (1926)
Eurybrachys tomentosa (Fab.)	Hemiptera: Eurybrachydae	Chatterjee (1933a)
Coccosterphus tuberculatus Motsch.	Hemiptera: Membracidae	Chatterjee (1939)
Nezara viridula L.	Hemiptera: Pentatomidae	Chatterjee (1939)
Petalocephala nigrilinea Walk.	Hemiptera: Cicadellidae	Chatterjee (1934)
Sarimanigro clypeata Melichar	Hemiptera: Fulgoridae	Chatterjee (1933b)
Coccids	Hemiptera	Chatterjee and Ayyar (1936)

Table 3. Putative insect vectors of phytoplasma diseases in forest trees of outside India

Host plant	Scientific Name	Order: Family	Reference
Paulownia spp.	*Halyomorpha halys* Stal	Hemiptera: Cicadellidae	Hiruki (1999)
Hovenia tomentella L.	*Hishimonus sellatus* (Uhler)	Hemiptera: Cicadellidae	Kusunoki et al. (2002)
Ulmus minor and *Ulmus pumila*	*Macropsis mendex* Lewis	Hemiptera: Cicadellidae	Carraro et al. (2004)
Ulmus sp.	*Scaphoideus luteolus* Van Duzee	Hemiptera: Cicadellidae	Hart (1978)
	Philaenus spumarius L.	Hemiptera: Cercopidae	
	Allygus atomarius Fab.	Hemiptera: Cicadellidae	
	Macrosteles quadrilineatus (*Fascifrons*)	Hemiptera: Cicadellidae	
Alnus glutinosa (L.)	*Oncopsis alni* (Schrank)	Hemiptera: Cicadellidae	Maixner and Reinert (1999)
Eucalyptus spp.	*Frankliniella occidentalis*	Thysanoptera: Thripidae	Marcone, 2015).
Fraxinus sp.	*Oncopsis alni* (Schrank)	Hemiptera: Cercopidae	(Maixner and Reinert, 1999).
	Orientus ishidae (Mastumura)	Hemiptera: Cicadellidae	
	Allygus mixtus Fab.		
	Allygus modestus Scott	Hemiptera: Cicadellidae	
	Psylla alni (Linn.)	Hemiptera: Cicadellidae	
		Hemiptera: Psyllidae	

of the vectors and/or reservoirs of phytoplasma-infected crop plants and weeds or management of phytoplasma spread within the tree by habitat management and the use of genetically disease resistant trees are the ideal ways in the management of phytoplasma diseases in the forest environment. Such habitat management can reduce the incidence and breeding of vectors. Maintaining conducive vegetation that can increase the incidence and abundance of natural enemies of vectors needs to be explored. Hence, more efforts should be made to determine those elements of the forest/tree environment that enhance the survival of natural enemies without supporting the population of vectors. There is a lot of scope to expand research on the management of phytoplasma diseases by identification of vector resistant phenotype/genotype of trees and applying the technology of tissue culture and genetic modification to produce plants with reduced phytoplasma titers or with insect resistance. Thus identification of real vectors is of paramount importance to understand the entire pathosystem of phytoplasma, for designing effective and economically and environmentally sustainable means of disease management.

CONCLUSION

Management of phytoplasma diseases in forest species is a great challenge as there is a huge lacuna existing in determination of real vectors and unavailability of resistant varieties/populations of trees. Therefore, critical knowledge not only on the actual vectors but also on their distribution, host range and mode of disease transmission are crucial for integrated management of phytoplasma diseases in forestry.

ACKNOWLEDGEMENTS

Authors acknowledge the projects funding by the National Authority CAMPA, Ministry of Environment, Forests and Climate Change, Government of India (GoI) under grant number No.75/2019/ICFRE (R)/RP/SFRESPE (CAMPA)/AICRP-3/Main File/28 and the National Medicinal Plant Board (NMPB), Department of Ayush, Government of India (GoI) under grant number Z.18017/187/CSS/R&D/KR-03/2019-20-NMPB-IV A. Authors also acknowledge the support and guidance provided by the Director General, Indian Council of Forest Research and Education (ICFRE) and the Director, Institute of Wood Science and Technology, Bangalore.

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(Manuscript Received: March, 2022; Revised: April, 2022;
Accepted: April, 2022; Online Published: May, 2022)
Online First in www.entosocindia.org and indianentomology.org Ref. No. e22039