

## Mycoplasma and allied diseases of forest trees in India and vector-host-pathogen interactions

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**Abstract.** Mycoplasma and allied diseases of forest trees in India have been described. MLO disease has been intensively studied in 'sandal' (*Santalum album* L.). Other forest trees showing typical MLO etiology are 'toon' (*Toona ciliata*), *Acacia catechu*, *A. mearnsii*, *Eucalyptus grandis* and *E. tereticornis*. Disease symptoms, collateral hosts, transmission tests with possible insect vectors and vector biology have been described for sandal spike disease. Reasons for contradictory results for sandal spike vectors have been discussed and possible lines of vector search for woody plants are described. Vector-host-pathogen interactions have been reviewed. During the feeding process, phloem cells are punctured, torn and disturbed by vectors. The hypertrophy of the plant cells around the site of puncture is caused by the action of injected salivary secretion. Vector borne procaryotic disease pathogens multiply within the vectors and circulate through a sequence of tissues and organs of vectors when the latent period of pathogen is long. These initiate both harmful and beneficial interactions within the vector. Possible lines of research to fill up the existing lucunae for insect vectors of mycoplasma and allied diseases of forest trees and vector-host-pathogen interactions have been discussed.

**Keywords.** Mycoplasma and allied diseases; forest trees; sandal spike; toon witches broom; *Eucalyptus* littleleaf; insect vectors; vector-host-pathogen interactions.

### 1. Introduction

Mycoplasma and allied diseases of plants are in general associated with chlorosis, reduction of leaf-lamina, shortening of internodes, floral phyllody and viriscence, stunting and witches' broom phenomenon. Prior to the epoch-making discovery of Doi *et al* (1967), all "yellows diseases" were considered as of viral origin on the basis of circumstantial evidence like their filterability and transmission by grafting. Mycoplasma and allied pathogens are now included in the class Mollicutes. The fundamental characteristics of Mollicutes are the absence of a distinct cell wall and their inability to synthesize the peptidoglycan polymer or its precursors (Maramorosch 1981). The Japanese workers (Doi *et al* 1967; Ishii *et al* 1967; Nasu *et al* 1967) were the first to demonstrate that these pathogens do not contain cell wall, are pleomorphic in outline and are present primarily in the sieve elements of plants, and the diseased plants respond to tetracycline treatment that leads to temporary remission of disease symptoms in plants. These pathogens have been found occurring in more than 200 plant species and numerous arthropods (Nienhaus and Sikora 1979). Among the forest trees, spike disease of 'sandal' in India and Paulownia witches' broom in Korea are well-known and well investigated. In addition to 'sandal', other forest trees exhibiting typical symptoms of mycoplasma and allied diseases are *Acacia catechu* at Marashettihally in Karnataka, *A. mearnsii* in Kodaikanal Hills of Tamil Nadu, *Eucalyptus grandis* in the Nilgiris of Tamil Nadu, *E. tereticornis* in plantations along the highways and canals in Haryana and Punjab, and in and around Jaipur in

Rajasthan, avenue trees of *Toona ciliata* at Kalyani township in West Bengal, and bamboos in some parts of India (Nayar 1973; Mukhopadhyaya *et al* 1977; Bakshi *et al* 1972; Rehill and Sen-Sarma 1982).

It has been established that these pathogens are often present in the insect vectors which transmit the disease agents from one plant to another. Therefore, pathogen-vector interactions are major components of epidemiology of these plant diseases. Further, pathogen-vector relationship assists in characterising the pathogen (Purcell 1982). Almost all the vectors so far implicated for transmission of mollicute plant pathogens belong to the insect order Homoptera which are primarily phloem feeder. Among the homopterans, the family Cicadellidae (suborder Auchenorrhyncha) has majority of the recorded vectors (Nielson 1968, 1979). A few vectors belonging to the family Psyllidae have also been recorded as transmitting MLO agency (Capoor *et al* 1967). The only aphid transmitted MLO disease is the grassy shoot disease (GSD) of sugarcane (Chona *et al* 1960; Singh and Shukla 1970).

In this paper an attempt has been made to evaluate the available information on the mycoplasma and allied diseases of some forest trees in India, the suspected and recorded vectors of these diseases, the biology and ecology of the vectors, transmission studies, and vector-pathogen-host interactions. Further, possible lines of future research have been outlined.

## 2. MLO diseases affecting forest trees

### 2.1 Sandal spike

Sandal (*Santalum album* L), well-known for its aromatic oil obtained from distillation of heartwood and roots, is affected by a devastating disease which is caused by a MLO agent (Dijkstra and Ie 1969; Hull *et al* 1969; Varma *et al* 1969).

This disease which was first observed in Coorg at the turn of the eighteenth century by McCarthy has now spread to many areas of Karnataka and Tamilnadu. Recently, the disease has been reported from Kerala in Marayoor area (Rehill and Sen-Sarma 1982 quoting Times of India 31.10.81). Muniappa *et al* (1980) carried out sandal spike disease survey in Karnataka during 1976–1979. Sampling methods followed were (i) systematic line plot sampling, (ii) stratified sampling and (iii) complete enumeration depending on the forest types. Based on this survey, they concluded that the average incidence of spiked sandal trees was 8.51%. Sen-Sarma *et al* (1978) have reported that using colour IR diapositive film and yellow (Wratten 12) filter from a height of 600 m (scale 1:7500), more than 2 m tall sandal trees could be distinguished from the surrounding plant species with 95% accuracy. Diseased plants could be identified by their dull pink colour (clear pink in healthy trees). IR black and white film could also be used profitably. According to Raychaudhuri and Varma (1980), this method has the potentiality of providing fairly accurate estimates of incidence of diseased trees in natural forests. The method is not time-consuming and tedious.

Sandal, being a semiroot parasite, is capable of parasitising a large number of tree species including herbaceous weeds (Bhatnagar 1965). Sandal trees are slow growing and oil can be extracted from trees having a minimum of 15–20 yrs growth. Natural stands of sandal primarily occur in bushy forests on rocky and gravelly soils (Sen-Sarma 1977b).

## 2.2 Disease symptoms

The most conspicuous symptoms in diseased trees are the reduction in leaf-lamina with a characteristic length breadth ratio, shortening of internodes resulting in spike-like appearance in affected branches, non-occurrence of fruits and flowers in infected branches with occasional development of viriscent or phylloid flowers in branches that are affected after initiation of flowering, necrosis of phloem tissues and shrivelling up or discontinuation of haustorial connections with the host plant/plants. Most of these symptoms develop during April to June which are also the months of plant growth. Disconnection with haustoria results in death within 1–5 yr after infection depending upon the age of the sandal tree. Some sandal trees mask the manifestation of external symptoms which can be forced out by pruning or pollarding (Sen-Sarma 1977b). A stain reaction for easy and field detection of suspected spike disease has been developed by Parthasarathi *et al* (1966). Free hand sections of healthy and spiked sandal twigs when stained with carbol thionin, giemsa or Mann's stain show clear differences between healthy and spiked twigs, Mann's stain giving the best differential staining. Diseased twigs stain violet and healthy twigs pink.

Proliferation of phloem tissues in diseased plants due to increased production of secondary phloem has also been recorded by Dijkstra and Van der Want (1970), and Hiruki and Dijkstra (1973a) and this results in development of necrotic areas mainly consisting of collapsed sieve-tubes (Narasimhan 1954). Higher cellulose content has been observed in sieve-tubes proximate to these necrotic areas in periwinkle, a collateral host of sandal spike (Hiruki and Dijkstra 1973b). The disease also increases the starch content of leaf parenchyma, pith and medullary rays in sandal (Narasimhan 1954). Fluorescence microscopy provides a very useful method in detecting infection. Even apparently healthy parts of diseased plants give a positive reaction for callose showing typical fluorescence, when stained with aniline blue (Dijkstra and Hiruki 1974).

## 2.3 Ecology of sandal spike disease

As is very well-known, sandal is a semiroot parasite. It can possibly survive for the first year without haustorial connections. But during the remaining period of its life, a sandal tree maintains haustorial connections as many as 200 times, notwithstanding stray records of haustoria-less plants surviving in nature as well as under laboratory conditions. A good deal of work was carried out during the thirties and forties to determine whether the host-plants impart any immunity to the sandal tree in respect of spike disease. Many workers (Mathew 1955; Muthana 1955; Sreenivasaya 1948) have recorded that incidence of disease is greater in areas where *Lantana camera* is the dominant host species. The possible explanations for this seem to be as follows: (i) The inadequate supply of nutrient to sandal, *Lantana* being a xerophytic type, (ii) increase in the incidence of insect vectors due to paucity of insectivorous birds (Sreenivasaya 1948) and (iii) greater build-up of inoculum, *Lantana* being a symptomless carrier of the disease pathogen (Nayar and Srimathi 1968).

The most important ecological factor of sandal spike pathogen is the occurrence of several collateral hosts in the natural sandal forests. The plants showing identical "yellows" symptoms are *Allamanda cathartica*, *Acacia* spp., *Catharanthus roseus*, *Dendrocalamus strictus*, *Dichrostachys cinerea*, *Dodonea viscosa*, *Scutia indica*,

*Stachytarpheta indica*, *Ziziphus oenoplea*, etc. (Rangaswami and Griffith 1941; Hull *et al* 1970; Ghosh *et al* 1977; Nayar 1973; Sen-Sarma 1977a). Experimental transmission of the disease agent from the diseased *Catharanthus roseus* and *Ziziphus oenoplea* to healthy sandal and *vice versa* through doddar was also achieved (Dijkstra and Lee 1972; Sen-Sarma 1977b).

#### 2.4 Vector transmission

Although the discovery of vectors of plant diseases is always a difficult task (Turner 1949; Tsai 1979), the difficulties are more acute for diseases of woody plants (Whitcomb and Coan 1982). The problems normally associated with the search of vectors of woody plants are long incubation period (Carter and Suah 1964) of the vector-borne pathogens in plants, irregular association of vector/vectors with the host (Kunkel 1933), low frequencies of acquisition (Jensen 1957) by vectors feeding on the diseased plants and low frequencies of transmission by inoculative insects (Howard and Thomas 1980). These difficulties primarily arise from the complexity of woody host plants that may have a few susceptible cells at any given time, and may have relatively fewer number of cells having high concentration of viable pathogenic agent. These facts are evidenced from the electron microscopic studies on wall-less prokaryotes diseases of woody plants (Breakbane *et al* 1972; Heinze *et al* 1972; Plavsic-Benjac *et al* 1972) showing low concentration and irregular distribution of causal agents (Jones *et al* 1974; Wilson *et al* 1972). The contradictory findings in respect of sandal spike vectors reported by various investigators may be considered keeping these in view. These difficulties were also encountered by many while searching for the vector of the coconut lethal yellowing disease (Tsai 1979; Howard and Thomas 1980).

That the sandal spike disease is vector-transmitted in nature was suspected by earlier workers when trap-plants in nature got infected (Sreenivasaya and Rangaswami 1934). Consequently, extensive studies on insect fauna associated with sandal were carried out and more than 200 species of possible insect vectors were screened for their ability to transmit spike disease. These studies conclusively proved that the possible insect vector/vectors of sandal spike disease frequent sandal plants only at night and the vectors are not very common in the sandal forest. Insects that produced spiked symptoms in transmission tests are *Moonia albimaculata* (Dover and Appana 1934), *Coelidia indica* (= *Jassus indicus*) (Rangaswami and Griffith 1941) and *Nephotettix virescens* (Shivaramakrishnan and Sen-Sarma 1978; Sen-Sarma 1981). Suspected spike-like symptoms were also obtained through *Coccosterphus tuberculatus*, *Nezera viridula* (Chatterjee 1940) and *Macrosiphum* sp (Dover and Appana 1934). Raychaudhuri and Varma (1980) while reviewing the transmission studies carried out by Shivaramakrishnan and Sen-Sarma (1978) are of the opinion that the investigations undoubtedly produced genuine infection in the test plants, though the technique was not sophisticated. On the contrary Muniappa *et al* (1980) reported negative results while repeating transmission tests using *M. albimaculata*, *C. indica* and *N. virescens*. They, however, used nymphal stage for acquisition feeding instead of adults. It may be noted that *C. indica* does not occur in South India (Nielson, *in litt*). Therefore, the identity of the so-called *C. indica* used by Muniappa *et al* (1980) is doubtful.

Vector transmission can be categorised into the following sequential phases (a) acquisition, (b) latency and inoculation (Purcell 1982). A vector may acquire the pathogen transovarially or by feeding on a infected plant. Transovarial transmission in

the leafhopper though postulated by Frazier and Posnette (1957) and Posnette and Ellenberger (1963) has been doubted by Chiykowski (1981). Thus, most acquisition takes place through feeding of the infected plants.

**2.4a Transmission with *Moonia albimaculata*:** Out of seven plants exposed to transmission studies with *M. albimaculata*, only one plant developed typical spike-like symptoms (Dover and Appana 1934). However, this was not confirmed by retransmission to healthy plants by grafting (Sen-Sarma 1981). Subsequent experiments by Rangaswami and Griffith (1941) indicate that spike-like symptoms might have manifested due to mass-feeding.

**2.4b Transmission with *Coelidia indica*:** Twenty nine adults released in batches of two or three were used in a wire mesh cage for acquisition feeding for 20 days. They were released to healthy sandal plants grown with *Acacia farnesiana* as the host plant for inoculation access. After about 60–90 days, only four plants exhibited typical disease symptoms. The disease was successfully transmitted from vector infected plants to healthy plants by leaf and bud grafting. All the insect-transmitted diseased plants died within 2–4 months (Rangaswami and Griffith 1941).

**2.4c Transmission with *Nephotettix virescens*:** During 1973–1975 Sen-Sarma tested *N. virescens* for transmission, as these insects were frequently observed in light-traps in spiked areas. Shivaramakrishnan and Sen-Sarma (1978) obtained infection in twelve plants out of fifteen tested using 60 viruliferous leafhoppers per test plant. Sandal test plants (3–5 years old) were grown in pots along with *Pongamia pinnata* (Syn. *P. glabra*) as the host plant. Vector-induced disease could be transmitted from these test plants to another set of test plants by bud grafting, thus confirming infection through the vector. The infection was further confirmed by stain reaction as developed by Parthasarathi *et al* (1966) and length-breadth ratio of spiked leaves (Iyenger 1961). *N. virescens* was bred under laboratory conditions on “Madhu” variety of paddy for the test purpose. After acquisition feeding for 48 hrs, the infective leafhoppers were allowed inoculation access for 7 days after which the test plants were transferred to an insect proof green house for further observations. A similar number of non-infective leafhoppers were used as control and allowed to feed on healthy plants for the same duration as in diseased plants after which they were released to another group of healthy sandal plants before the plants were transferred to the insect proof green house. The experiments were repeated several times and in many cases the vector fed plants developed the spike symptoms and died within 4–5 months after external manifestation of the symptoms. None of the infected plants recovered. It is worth noting that adults of *N. virescens* were also successfully used in transmitting the MLO agents of diseased *Catharanthus roseus* to healthy sandal seedlings and *vice versa* (Shivaramakrishnan, *in litt*). Nayar and Ananthapadmanabha (*in litt*) claim to have been able to transmit the disease agent cultured in synthetic medium *via* adults of *N. virescens*. It seems that the latent period of the pathogen within *N. virescens* is either very short or practically non-existent. This, however, does not imply non-multiplication of disease agent in its vector (Purcell and Finlay 1979). Failure of Muniappa *et al* (1980) to obtain disease transmission *via N. virescens* may be attributed to using nymphs, long latent period and very young test plants. It is pertinent to mention that Sreenivasaya and Rangaswami (1934) reported constant association of agricultural operations with the primary site of disease outbreak.

### 2.5 Biological observations on sandal spike vectors

**2.5a *Moonia albimaculata*:** In Karnataka, the species has been recorded from the districts of Bangalore, Bidar, Bijapur, Belgaum, Chikmagalur, Dharwar, Hassan, Kodagu, Kolar, Mysore, Tumkur and North Kanara (Muniappa *et al* 1980). It also extensively occurs in sandal growing areas of Tamilnadu. In southern Karnataka, the species occurs more abundantly. Though it is commonly found on sandal trees, it does not breed on them. Other host-plants from which the species has been collected are *Dodonea viscosa*, *Terena asiatica*, and *Pterocarpus indicus* (Sen-Sarma 1981). It has probably three overlapping generations in a year. Eggs are inserted on the edges of leaves of its host-plants by making slits in plant tissues.

**2.5b *Coelidia indica* (= *Jassus indicus*):** Muniappa *et al* (1980), in a detailed survey of sandal forests of Karnataka, have collected the so-called *C. indica* from 92 forests out of 319 surveyed. This species is very common on *Zizyphus jujuba* and *Dodonea viscosa*, although the insects also occur on both healthy and diseased sandal. This species is also reported to be very common in Jawalagiri in Tamilnadu (Rangaswami and Griffith 1941). Eggs are laid in small slits on tender leaves of breeding hosts. Life-history is completed within 12–14 weeks. Longevity of adults is about three months (Sen-Sarma 1981).

**2.5c *Nephotettix virescens*:** Muniappa *et al* (1980) have carried out extensive studies on duration of different instars and longevity of adults on paddy, periwinkle, and sandal. Maximum nymphal duration and adult longevity were observed on paddy leaves. This species is highly phototropic and, therefore, remain hidden during day time. Its biology and ecology on rice have been rather extensively studied. It breeds only on succulent green grasses near tanks, rivers, canals, etc in nature. It is common in paddy fields throughout India. Its population in sandal ecosystem starts declining progressively by the middle of February until the minimum is reached in the month of May, as evidenced by light-trap collections (Sen-Sarma 1981). The population build-up commences in June and reaches the peak in October–November.

### 3. Witches' broom disease of toon (*Toona ciliata*)

Toon is an important forest species in India. It is grown both in plantations and as avenue trees. Recently, toon, grown as avenue trees at Kalyani Township in West Bengal, has suffered witches' broom disease. The disease first reported by Mukhopadhyaya *et al* in 1977 in about 33% of the trees has now almost completely wiped out the toon trees from the Kalyani Township. The diseased trees exhibited symptoms like severe reduction in leaf-lamina, shortening of internodes, extensive proliferation of axillary buds leading to witches' broom, cessation of flowering and fruiting. Initially, the symptoms appear only in a few branches of the affected tree, gradually spreading all over the tree. The diseased tree does not recover and die within 4–5 years of the initial manifestation of external symptoms. The leafhoppers belonging to the genus *Empoasca* occur in large number on both healthy and diseased trees which tends to suggest their possible role as the vector of the disease. *Empoasca* spp are known as vectors of MLO disease of plants (Bindra 1973). However, no

systematic study to establish the vector/vectors of witches' broom disease of toon has been carried out so far.

#### 4. MLO disease of *Acacia* spp

Nayar (1973) reported occurrence of typical 'yellows' symptoms in *Acacia catechu* in Marashettihally in Karnataka and *A. mearnsii* in Kodaikanal Hills in Tamilnadu. However, electron microscopic examination of diseased material so far conducted did not give positive results. Perhaps examination of a large number of typically diseased material may prove confirmatory (Rehill and Sen-Sarma 1982).

#### 5. Little-leaf disease of *Eucalyptus*

Reduction of leaf-lamina has been reported in *Eucalyptus grandis* seedlings in the Nilgiri Hills (Tamilnadu). EM examination of diseased leaves showed tentative evidence of MLO disease (Nayar 1973).

Sporadic chlorosis of leaves accompanied by shortening and narrowing of leaf-lamina and proliferation of axillary buds with witches' broom growth of shoots has been observed in *E. tereticornis* trees raised along highways and canal banks in Haryana and Punjab. Similar symptoms have also been observed in *E. tereticornis* growing around Jaipur in Rajasthan. The symptoms are suggestive of MLO etiology. EM examination of thin sections of diseased leaves revealed presence of typical MLOs in sieve elements (Maramorosch *et al* 1982).

#### 6. Vector-pathogen-host interactions

Not much information is available on the subject in respect of vector-pathogen-host interactions of MLO agents of forest trees. However, some information available in literature may be applicable to these as well, as discussed below:

##### 6.1 *Vector-host interactions*

Vectors of MLO diseases are primarily phloem feeders. During the feeding process, the phloem cells are punctured, torn and distorted. The puncturing is also accompanied by secretion of a protein or some pectinate substance apparently from the salivary glands. This secretion forms a sheath around the stylets (Smith 1933). According to Medler (1941) the injury to plants caused by *Empoasca fabae* is a combination of the feeding in the vascular tissues and action of a specific compound which is injected during the feeding process. The hypertrophy of the affected plant cells around the site of puncture is attributed to injection of salivary secretion. The mechanical injury caused during puncturing disorganises the plant tissues which often results in clogging and isolation of vascular bundles (DeLong 1971).

##### 6.2 *Vector-pathogen interactions*

In sandal spike disease, disease transmission can take place through the haustorial connections, notwithstanding low percentage (5-7%) (Coleman 1917). However,

transmission in majority of cases occurs through the agency of insect vectors (Sen-Sarma 1977b). In such cases, the insect vectors act as the intermediary hosts of the disease agency. It has been proved almost conclusively that pathogens multiply within the vector-body (Maramorosch 1952). The relationships between vector and pathogen are most intimate but complex (Purcell 1982). Unlike vector-borne diseases of human and animals, most wall-less procaryotic pathogens of plants have broad vector spectrum, as is suspected in sandal spike disease. The vector-pathogen interactions have been studied in depth in aster yellows in USA. The discovery that, in addition to the main vector, *Macrostelus fascifrons*, many species of leafhoppers in the subfamily Deltacephalinae could transmit California strain of aster yellows is a pointer in this direction (Severin 1945, 1947). Important aspects of vector-pathogen relationship are the information on acquisition threshold, optimum acquisition period, latency and inoculation threshold. In *N. virescens* indirect evidence shows that it has a very short latency period in contrast to long latency period in most vectors of yellows agents. However, short latency period does not necessarily imply non-multiplication and non-circulation of the MLO agents within the vector. Where the latent period is long, pathogens are required to multiply and circulate through a sequence of tissues and organs within the vector (Purcell 1982). Detection of MLO agents within gut tissues, fat body, mulpighian tubules, salivary glands are evidences for multiplication and circulation of yellows agents within the vectors (Townsend *et al* 1977). Plant pathogenic wall-less procaryotes are reported to initiate interactions within the vectors, some harmful and some beneficial (Maramorosch and Jensen 1963; Whitcomb and Williamson 1979).

#### 7. Possible lines of future research

The most important lacuna in our knowledge of vector-borne wall-less procaryotic diseases of forest trees is that the possible vector/vectors has/have not been conclusively discovered. It is a matter of great regret that the excellent work carried out between 1933–1941 on vector transmission of sandal spike disease was discontinued for about three decades. Recent attempts in this direction has also been vitiated due to lack of sophisticated techniques. Further, failure to cultivate the culture of the disease pathogen under laboratory conditions further accentuated the difficulties of the Forest Entomologists. Blind passage technique for vector searches as developed by Whitcomb and Coan (1982) seems potentially useful in the search for vectors of MLOs diseases of woody plants and this technique should be given a fair trial. Many lacunae exist in our knowledge on vector-host interactions and vector-pathogen interactions. Multiplication and spread of plant pathogenic agents in the vectors can possibly be best studied by employing high resolution autoradiography (Gouranton and Maillet 1973). Our knowledge of effect of these plant pathogens on the insect vectors should be advanced by resorting to simple techniques like studying different insect organs separately employing histopathological, histochemical and biochemical methods. Further, environmental influences on disease progress after inoculation should form an integral component in the vector transmission studies either in the field or in the laboratory (Purcell 1982).

### Acknowledgement

The author sincerely thanks Prof. K Maramorosch of Waksman Institute of Microbiology, Rutgers (USA) and Dr R F Whitcomb, Plant Disease Vector Laboratory, Beltsville (USA) for supplying some literature on the subject.

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