

Plant Mollicutes and Bacteria-like Organisms

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INTRODUCTION

In recent years many new groups of pathogens causing diseases in plants have been identified. Most of these pathogens are associated with virus-like diseases with which no typical virus could be identified earlier. The most important 'new group' of pathogens is the mycoplasma-like organisms (MLOs) which cause a large number of economically important yellows type of diseases (YTDs) in plants in India and other parts of the world (Varma and Rajamannar 1989). Until 1967—when a team of Japanese workers demonstrated the association of MLOs with YTDs (Doi *et al.* 1967, Ishiie *et al.* 1967), these diseases were considered to be viral due to filterability of the causal pathogens through bacteria-proof filters, and transmission through insect vectors in a persistent manner. In India too sandal spike disease (like other YTDs) was considered to be viral (McCarthy 1903) till the late 1960s when the association of MLOs was demonstrated independently by three groups of workers (Dijkstra and Ie 1969, Hull *et al.* 1969, Varma *et al.* 1969). Since then, MLOs have been reported to be associated with a large number of diseases (Varma and Rajamannar 1989).

Some of the YTDs are caused by Spiroplasmas (Bove *et al.* 1973) which resemble Mycoplasmas and belong to the same class of Mollicutes (Varma 1977). Mycoplasmas, which are saprophytic or cause diseases in animals, and all Spiroplasmas can be cultured on synthetic nutrient media. So far, no YTD-causing Mycoplasma has been cultured on synthetic medium making their identity tentative, hence the name MLO. Similarly, bacteria-like organisms (BLOs), which cause important diseases like citrus greening, cannot be cultured on synthetic medium.

In India YTDs like sandal spike (McCarthy 1903) and little leaf disease of brinjal (Thomas and Krishnaswami 1939) were known as 'viral

diseases'. In 1969 the association of MLOs (Varma *et al.* 1969) and recovery in symptoms by tetracycline antibiotic treatment (Anjaneyulu and Ramakrishnan 1969, Raychaudhuri *et al.* 1972) suggested mycoplasmal etiology of these diseases. The possibility of chemotherapy to control YTDs, in contrast to the viral diseases which do not respond to any chemotherapeutic treatment, has created interest and active research on YTDs has been undertaken at several agricultural and traditional universities and research institutes.

For historical reasons, research on MLOs and BLOs has remained mainly in the hands of virologists not only in India but all over the world. An equal participation of bacteriologists would have been advantageous, but it is always difficult to leave 'green pastures' to work on difficult organisms like fastidious prokaryotes. The major centres of research on MLOs and BLOs are: Indian Agricultural Research Institute (IARI), New Delhi; Central Potato Research Institute (CPRI), Shimla; University of Agricultural Sciences, Bangalore; Central Plantation Crop Research Institute, Kayangulam; Indian Institute of Horticultural Research, Bangalore; Department of Botany, University of Rajasthan, Jaipur. All these centres started working on these organisms in the late sixties and the early seventies.

TAXONOMIC STATUS

Conscious of the need to satisfy Koch's postulates to confirm mycoplasmal etiology of YTDs, plant pathologists the world over attempted to isolate and characterize plant-inhabiting MLOs. No one succeeded but, in the process, came to light new groups of microbes—the spiroplasmas which could be cultured on artificial medium, and the fastidious BLOs. *Mycoplasma* and *Spiroplasma* belong to the class Mollicutes and order Mycoplasmatales. Mollicutes, as the name suggests (moll=soft, cute=skin), are the organisms without cell wall. Their new class within lower protista was created by Edward and Freundt (1967) to include organisms earlier placed in the tenth order of the class Schizomycetes, named as Mycoplasmatales. These organisms are unable to synthesize peptidoglycan and its constituents muramic acid and diaminopimelic acid, are pleuromorphic with a tendency to penetrate into the depth of solid medium forming 'fried egg'-shaped colonies; resist antibiotics which prevent polymerization of cell wall precursors—require sterol for growth; have small genome size of less than 1×10^9 daltons and G + C content of DNA between 23 and 45 per cent (Varma 1977).

Mollicutes are mostly saprophytes; some cause diseases in man, animals and plants. A mollicute (*Mycoplasma*), popularly known as pleuropneumonia-like organism (PPLO), was first isolated from pleuropneumonia-affected bovine (Nocard and Roux 1898). In animals mollicutes are generally extracellular parasites, whereas in plants

they are exclusively intracellular parasites normally restricted to sieve tubes. Some *Spiroplasma*, however, also occur extracellularly in floral nectaries. In ultrathin sections, *Mycoplasma* and *Spiroplasma* are difficult to distinguish. The plant-infecting mollicutes which can be grown on artificial medium and have spiral filaments have been identified as Spiroplasmas; the identity of others remains tentative and are referred to as Mycoplasma-like organisms (MLOs). In India, so far, free-living or plant-parasitic *Spiroplasma* has not been identified although, judging from their ubiquitous nature, it is a matter of time before these are detected. In other geographical areas, three species of *Spiroplasma* cause plant diseases; *S. citri* causes citrus stubborn and horse radish brittle root (Raju *et al.* 1981), *S. kunkelii* causes corn stunt (Chen and Liao 1975), and *S. phoeniceum* causes a disease in periwinkle (Bove 1984). In contrast, over 300 plant diseases are associated with MLOs, many of these occur in India (Varma and Rajamannar 1989).

Fastidious bacteria-like organisms (BLOs) can be distinguished from mollicutes by the presence of a true cell wall. These are also, occasionally, referred to as Rickettsia-like organisms but this name is inappropriate as a close relationship has not been established with *Rickettsia*. Two types of BLOs cause diseases in plants: Pierce's disease of grapevine (Davis *et al.* 1978), and phony disease of peach (Davis *et al.* 1981) which are xylem-inhabiting. Those BLOs which cause diseases like citrus greening (Lafleche and Bove 1970) and grapevine yellows (Kuppers *et al.* 1975) are phloem-inhabiting. In India only phloem-inhabiting BLOs have been identified.

YELLOW TYPE OF DISEASES (YTD)

Nearly 100 yellows type of diseases (YTDs) occur in India but the important ones are sandal spike, coconut root wilt, citrus greening, sesamum phyllody, brinjal little leaf, rice yellow dwarf, and potato purple top roll (Raychaudhuri *et al.* 1977; Varma and Rajamannar 1989). These diseases cause enormous economic losses. Sandal spike resulted in a loss of Rs 3 million in 1969 alone (Raychaudhuri and Varma 1980), and coconut root wilt threatens the entire coconut industry of Kerala with an estimated annual loss of 968 million nuts (Solomon *et al.* 1989). Due to the high incidence of phyllody disease, the cultivation of sesamum (*Sesamum indicum*) in north-west India is nearly abandoned (Verma and Daftari 1985). Little leaf disease of brinjal (*Solanum melongena*) causes nearly 50 per cent loss annually (Thomas and Krishnaswami 1939).

Sandal Spike. Sandal is a semi-root parasite which has been grown in India since the second century BC. It is well-known for its fragrance

and heartwood. Infection by MLO causing spike disease, results in reduction in the size of the leaves and shortening of internodes, giving an appearance of a spike to the affected branches. There are aberrations and degenerative changes in the embryo sac and the embryo in partially-spiked trees. However, in advanced stages of disease, cessation of flower and fruit production is common (Nayar *et al.* 1987). The disease is confined to south-east Karnataka and north-west Tamil Nadu. Several factors such as fire, dense stand, the presence or absence of certain hosts of sandal, and weather conditions affect the distribution of spike disease. The disease incidence in southern Karnataka ranges from 1 to 55 per cent with an average of 8 per cent (Rao and Muniyappa 1988).

The age and size of the sandal plant do not affect its susceptibility but generally the incidence is higher in areas where *Lantana camara* is the dominating host species of sandal (Raychaudhuri and Varma 1980, 1988). Several species of insects have been claimed to be the vector of the causal MLO but the validity of the transmission remains doubtful (Raychaudhuri and Varma 1988). A little leaf disease is common in species of *Stachytarpheta*, *Dendrocalamus*, *Randia*, *Dichrostachys*, *Acacia*, *Eucalyptus*, *Barleria* and *Scutia* growing near sandal wood with spike disease. MLOs are also associated with some of these collateral hosts of sandal (Nayar and Ananthapadmanabha 1977). It is not known whether the MLOs associated with the little leaf disease of these hosts are similar to the MLOs associated with spike disease of sandal. The causal MLO, however, can pass through haustorial connections from the host of sandal to sandal and *vice versa*. Coleman (1923) and Rangaswami and Griffith (1939) could not transmit the disease-causing agent from *Datura stramonium*, *Dodonea viscosa*, *Vinca rosea* (= *Catharanthus roseus*) and *Ziziphus oenoplea* to sandal; all of these are naturally affected with similar diseases. For the effective control of this devastating disease, it is desirable to encourage the hosts of sandal which impart resistance, and eradicate those which increase susceptibility (Raychaudhuri and Varma 1988). The production of spike-free plants *via* somatic embryogenesis and breeding for resistance (Sita and Ram 1981) will be useful in the long-term management of the disease. Nayar (1984) isolated a toxin from spiked sandal wood and injected it into healthy seedlings of sandal and *Stachytarpheta jamaicensis* but leaf abnormality was not observed in either species suggesting that the toxin is not involved in the disease syndrome.

Coconut Root (Wilt) Disease. It is the most serious disease of coconut palms in the country and occurs in patches in all types of soil, especially along the water canals. The leaves of affected plants become flaccid giving a 'rib'-like appearance, which gradually turn yellow and

wilt. The growth rate is reduced which results in stunting, poor root system, reduced yield, and gradual death of plants. A severe epidemic occurred in Travancore (now Kerala) in 1882 after widespread floods when the land remained water-logged for a long period; since then the disease has been spreading slowly and steadily (Lal 1966). The etiology of this disease has been long debated, due to conflicting reports on its causative agent. The MLOs have been observed consistently associated with the disease, and these are transmitted by insects *Stephanitis typica* and *Proutista moesta* (Solomon *et al.* 1989). However, contrary to expectations, the disease has not satisfactorily responded to the treatment with tetracycline antibiotics. Yellow leaf of arecanut and tatipaka disease of coconut are also similar diseases. Further investigations are necessary for establishing the etiology of these important diseases.

Citrus Greening. This disease is one of the major constraints in citrus production in the country (Fraser *et al.* 1966, Capoor *et al.* 1967, Raychaudhuri and Ahlawat 1982). The causal organism of the disease has been established as a gram-negative bacterium (BLO) (Naidu and Govindu 1981). Most of the citrus species and its relatives are susceptible to this pathogen. The main symptoms of the disease are chlorosis of interveinal areas, mottling and smalling of leaves, and die back of twigs. The secondary growth results in upright, short and weak bushy shoots. Chronically affected trees show various degree of mottling on the leaves. Occasionally, circular green spots appear on yellow tissue in the younger leaves as green islands, suggestive of zinc deficiency. The fruits show conspicuous sun blotching. The primary spread of the disease is through budwood, and the secondary spread through the citrus psylla, *Diaphorina citri* (Capoor *et al.* 1967). Certain root stocks like sweet lime and pummelo are comparatively tolerant. The disease can be managed by the use of healthy planting material and timely control of the insect vector (see Raychaudhuri and Ahlawat 1982).

Sesamum Phyllody. Phyllody is the most devastating disease of sesamum (*Sesamum indicum*) and can cause heavy losses if conditions are favourable for the multiplication of its vector. The disease is characterized by vein clearing of the leaves, production of phylloid flowers, shortening of internodes, abundant axillary growth of the buds, and reduction in leaf size. The affected plants do not set any pod and result in total loss in yield. The disease is transmitted in nature by a leaf hopper *Orosius albicinctus*, in a persistent manner (Sahambi 1970). The causal MLO can infect 90 plant spp. belonging to 38 genera in 12 families. The important hosts are *Crotalaria juncea*, *Brassica campestris*, *B. rapa*, *Cicer arietinum*, *Trifolium* spp. and many ornamental plants (Sahambi 1970). Late sowing reduces the incidence of the disease (Raychaudhuri and Nariani 1989).

Brinjal Little Leaf. Little leaf disease of brinjal was observed as early as 1939 by Thomas and Krishnaswami. The main symptoms on brinjal are the reduction of leaf size, excessive growth of axillary buds, and shortened internodes resulting in a bushy appearance of the affected plants. The flowers are phylloid and there is a total loss in yield. The disease is transmitted by a jassid, *Hishimonas phycitis*. The causal MLO (Varma *et al.* 1969) also infects other solanaceous species like tomato, potato and tobacco. The symptoms of the disease are suppressed by treatment with tetracycline antibiotics (Varma *et al.* 1975).

Rice Yellow Dwarf. This disease is prevalent in Orissa, Bihar, West Bengal and Andhra Pradesh, and causes epidemics in favourable years. The leaves of affected plants develop general chlorosis with pronounced stunting and profuse tillering. The disease is lethal to the plants infected at an early stage. The MLOs are associated with the disease and it is transmitted by the green leaf hopper, *Nephotettix impicticeps*. Most of the rice varieties are susceptible to this disease (see Raychaudhuri *et al.* 1977).

Grassy Shoot Disease of Sugar Cane (GSD). GSD was first recorded in 1955 (Vasudeva 1955), and has been reported from various parts of the country, under different names. Rane and Dakshindas (1962), however, have shown that the disease referred to as grassy shoot (Chona *et al.* 1960), or yellowing (Kulkarni 1962), or albino (Kirtikar and Singh 1958) are one and the same disease. Under favourable conditions, up to 56 per cent incidence has been recorded (Bhargava *et al.* 1966). The disease is prevalent in Andhra Pradesh, Bihar, Uttar Pradesh, Madhya Pradesh, Tamil Nadu and Maharashtra. GSD is characterized by the production of numerous thin tillers with short internodes from the base of the affected plants. A bunch of tillers bears pale-yellow, thin and narrow leaves. If the crop becomes infected at an early stage, the loss may be up to 100 per cent. The MLOs are associated with the disease (Rishi *et al.* 1973). Tetracycline antibiotic treatment results in temporary remission in disease symptoms (Singh *et al.* 1973). A complete control of GSD—both in main crop and ratoon crop—can be obtained by exposing the seed canes to hot air in an air-tight chamber at 54°C for eight hours (Singh *et al.* 1973). A mixed infection of sugar cane mosaic and grassy shoot also occurs (Bhargava *et al.* 1966).

Apple Rubberywood. This disease is prevalent in Himachal Pradesh (Dhingra and Ahlawat 1973) and Kumaon hills, on several varieties of apple (Bhargava and Bist 1960). The MLOs are associated with this disease (Beakbane *et al.* 1971). The characteristic symptom

is the rubbery texture of stem. The affected branches are extremely flexible and droop. Most of the apple varieties are susceptible to this disease which mainly spread through infected budwood. Natural vectors of the MLO are not known.

Peach 'X' Disease. The peach 'X' disease has been reported from north-eastern India on peach (Ahlawat and Chenulu 1979) and from Kulu valley on almonds (Ahlawat *et al.* 1984). The disease is characterized by numerous shoots growing upright with varying degrees of anthocyanosis, die back and general decline. The leaves on affected branches roll upward and fall prematurely and the affected trees die within a few years of infection. The disease is transmitted by a leaf hopper vector, *Exitianus fusconervosus*, to celery and periwinkle. Remission of disease symptoms has been observed by tetracycline therapy (Ahlawat *et al.* 1984).

Cotton Stenosis/Small Leaf. This disease has been reported from Maharashtra (Uppal *et al.* 1944) and is spreading to other parts of the country (Capoor *et al.* 1972). The leaves of the diseased plants become variously lobed, malformed, and of different shape and size. Foliar outgrowths as enations are produced on veins on the undersurface of the diseased leaves of affected American cottons. Boll formation is considerably reduced. MLOs are associated with the disease and also respond to tetracycline antibiotic treatment (Capoor *et al.* 1972).

Potato Purple Top Roll. This disease is of common occurrence in potatoes grown in the north-west hills of India (Nagaich and Giri 1973). The characteristic symptoms are chlorosis, purple pigmentation and rolling of basal parts of young leaflets. Proliferation of axillary shoots, swelling of nodes, and development of occasional aerial tubers are also associated with the disease. Unusually, the mother tuber remains hard till harvest, whereas the progeny tubers produce hairy or spindly sprouts.

The association of MLOs with purple top roll disease has been demonstrated (Nagaich *et al.* 1974). The disease spreads through infected tubers and leaf hopper vectors, namely *Orosius albicinctus* and *Alibroides olrauidanus* (Singh and Nagaich 1977).

The disease can be effectively managed by the use of healthy seed tubers, roguing of diseased plants in the field, and removal of spindly sprout tubers from storage seed. Tetracycline therapy has been successful in suppressing the symptoms of the disease (Nagaich *et al.* 1978).

Eucalyptus Little Leaf. In recent years, the incidence of this MLO disease has dramatically increased (Sharma *et al.* 1983). The diseased

plants develop severe die back resulting in the loss of trees. Further studies are required to understand this problem.

CHARACTERISTICS OF YELLOWS TYPE OF DISEASES (YTDs)

A large number of yellows type of diseases affect other cultivated and wild plant species (see Raychaudhuri *et al.* 1977, Varma and Rajamannar 1989). These diseases also affect important crops like grain legumes (Varma *et al.* 1978), oil-seeds, vegetables (Raychaudhuri *et al.* 1977), fruits and forest trees (Varma *et al.* 1991), but so far their incidence has remained low. These and other YTDs of wild plants need further investigations to fully understand their epidemiological significance.

Potato purple top roll is a serious YTD of potatoes in the north-western hills of India (Nagaich and Giri 1973), with which association of MLOs has been demonstrated (Nagaich *et al.* 1974). The disease spreads through infected tubers and leaf hopper vectors (Singh and Nagaich 1977), and tetracycline treatment suppresses symptom development (Nagaich *et al.* 1978). The disease is effectively managed by the use of healthy seed tubers. Another important YTD is the cotton stenosis (Uppal *et al.* 1944). MLOs have also been associated with this disease and respond to the tetracycline antibiotic treatment (Capoor *et al.* 1972). Apple rubberywood and peach 'X' diseases are common in temperate fruit-growing areas of Himachal Pradesh and Kumaon hills (Bhargava and Bist 1960, Ahlawat and Chenulu 1979). These diseases cause decline in production. The MLOs are associated with these YTDs (Beakbane *et al.* 1971) and they also respond to tetracycline antibiotic treatment (Ahlawat *et al.* 1984). In recent years MLO disease-causing little leaf in *Eucalyptus* has also increased considerably (Sharma *et al.* 1983).

For sometime the ratoon stunting disease of sugar cane was also considered to be a YTD, but now it is known to be caused by a xylem-limited bacterium, *Clavibacter xyli*. This disease was first recorded in India in 1956 (Chona 1956) and it occurs in most of the sugar cane-growing states (Bhargava *et al.* 1966). The affected canes are stunted and thinner than the normal ones. Internal stalk discolouration is characteristic of the disease which can be managed by hot water treatment at 50°C for three hours (Shukla *et al.* 1974).

Diagnosis. At the advanced stages of infection, YTDs are easily recognizable by their distinctive symptoms. This, however, requires further confirmation by histopathology, electron microscopy and chemotherapy. Non-cultivability of the causal MLOs and BLOs make specific diagnosis difficult. Serodiagnosis and nucleic acid

hybridization provide useful specific diagnosis but require monoclonal antibodies and cDNA probes. In India these methods are still at the initial developmental stages.

Symptoms. The characteristic symptoms produced by the YTDs can be grouped into four broad categories, that is yellows type, stolbur type, witch's broom type, and phyllody type (Varma and Rajamannar 1989). In yellows type the affected plants develop short internodes, leaf yellowing and smalling, and decline as in citrus greening, and sandal spike diseases (Raychaudhuri and Varma 1989). Stolbur type results in reduced apical growth, stunting, thickening of stem, smalling of leaves, and virescence as in tomato stolbur and brinjal little leaf diseases (Varma *et al.* 1975). Witch's broom type, caused by excessive proliferation of axillary buds, is common in *Parthenium*, cowpea and *Zizyphus* (Varma, Sang *et al.* 1974, 1975, Pandey *et al.* 1976). Typical symptoms are the phyllody type in which floral parts become converted to leafy structures as in sesamum and sunn hemp phyllody (Bose and Misra 1938). The latter two categories of symptoms are characteristic of YTDs, although some fungal diseases like mango malformation (Varma, Lele *et al.* 1974) also shows similar symptoms. Some YTDs result in reduced lignification of xylem vessels leading to drooping of stems, as in apple and citrus rubberywood diseases (Ahlawat and Chenulu 1979).

Physiological Changes. YTDs cause considerable changes in the physiology of the affected plants. Disintegration of haustoria in diseased sandal plants affects mineral uptake. The amount of minerals decreases under pathogenesis in leaves, but iron increases in stem and roots (see Raychaudhuri and Varma 1988). The change in calcium : iron ratio in spiked sandal can be used for diagnostic purposes (Iyengar 1972). Low iron content and high chlorophyllase activity results in general chlorosis of leaves of sandal under pathogenesis (Parthasarathi *et al.* 1976). Poor transport of sugars due to phloem necrosis in diseased plants results in high starch content in YTDs (see Raychaudhuri and Varma 1988). Changes like reduction in chlorophyll content, photosynthesis and photorespiration rates, and increase in phenols and respiratory rates due to MLO infection are common (Dhumal and Nimbalkar 1982, Shukla *et al.* 1984) but have limited utility in diagnostics.

Further studies are necessary, particularly for the identification of vivo-toxins as produced by spiked sandal (Gurumurthi *et al.* 1979).

Histopathology. Detectable anatomical changes occur in plants affected with YTDs. The leaves of sandal affected with spike disease

have cellular hypertrophy, accumulation of starch, and poorly developed vascular system; the diseased plants do not produce heartwood (Butler 1903). Proliferating intracellular inclusion bodies also occur in epidermal cells (Dijkstra 1968). The origin of these intracellular inclusions due to MLO infection is uncertain (Raychaudhuri and Varma 1988). The phloem in diseased plants proliferates and develops necrotic areas due to collapsed sieve tubes (Ghosh *et al.* 1974). Infection also results in the deposition of callose in phloem cells which can be detected by autofluorescence or staining with fluorochromes like aniline blue (Mittra and Gupta 1984). Detection of MLOs associated with brinjal little leaf is most specific and sensitive using DAPI stain (4, 6-diamidino-2-phenylindole-2HCL) (Singh and Mittra 1989).

Electron Microscopy. Electron microscopy provides direct evidence of the association of MLOs and BLOs with YTDs (Varma *et al.* 1982). In an ultrathin section of sieve tubes of diseased plants, MLOs look nearly spherical, diameter 50 to 1000 nm, mostly 250 to 500 nm (Varma *et al.* 1975). Occasionally, they are also present in phloem parenchyma cells (Raychaudhuri *et al.* 1970). Their pleomorphism is more obvious in longitudinal sections showing apparent movement of MLOs from one sieve cell to another through sieve pores. Each MLO is bounded by a triple-layered membrane of 7 to 12 nm thickness; the cells contain unbounded nuclear region, ribosomes and cytoplasmic granules but there is no membranous organelle (Varma *et al.* 1975, Garg *et al.* 1989).

BLOs can be distinguished from MLOs by their thicker (21 to 31 nm) envelope than those of MLOs (Varma 1977). They are bounded by a double trilaminar membrane which often appears to be separated by an electron-luscent zone.

Serodiagnosis. Rajamannar and Varma (1989) could purify the antigen from phloem tissues isolated by enzyme treatment, that produced antiserum which gave specific precipitin bands against solubilized antigen (brinjal little leaf MLO) in gel diffusion tests and also gave specific reactions in ELISA. Purification of antigen by differential centrifugation is not very useful as a larger proportion of host contaminants remain in such preparations resulting in non-specific serological reaction.

Garg *et al.* (1989) obtained moderately intense reaction in ELISA with extracts from hairy sprouts of tubers collected from plants infected with marginal flavescence and antiserum specific to *Spiroplasma citri* obtained from the U.S.A. These results suggest probable involvement of *S. citri* with marginal flavescence in potato, but it requires confirmation.

Culture on Synthetic Medium. MLOs or BLOs have been isolated from plants affected with YTDs like citrus greening, clover phyllody, sandal spike, sugar cane white leaf, etc., but none of these has been characterized sufficiently to meet various criteria (Varma 1977) to confirm their infectivity.

Ghosh *et al.* (1971) cultured the citrus greening organism—a BLO—on a simple medium containing PPLO agar/broth and rabbit serum. The cultures could be grown at pH 6.0–7.8 at 25° to 35°C. PPLO medium supplemented with bacterial culture filtrates, serum, nucleic acids and cholesterol support good growth, but plant extracts were poor supplement (Ghosh *et al.* 1975). The organism could cause disease in *Citrus sinensis* seedlings inoculated through the vector *Diaphorina citri* made infectious by injecting the cultured organism (Nariani *et al.* 1975). In these experiments, however, the culture was not sufficiently diluted to eliminate retention of infective units from the original plant extract used as inoculum for the culture of the organism. The characteristics of organism were similar to mycoplasmas, and it was inhibited by tetracycline antibiotics but not penicillin suggesting doubtful identity of the organism.

The MLOs associated with sandal spike and arecanut yellowing are also reported to be brought to culture (Nayar and Ananthapadmanabha 1970, Nayar 1971), but there has been no follow up of these studies. The cultures grew maximally at 37°C; this temperature favours animal mycoplasmas but is not expected to favour growth of MLOs as the diseased plants maintained at 37°C continuously recover.

Natural Spread. Natural spread of all YTDs is through insect vectors as reported earlier for sandal spike, by the temporary prevention of its spread by clearing a belt of sandal and *Ziziphus oenoplea* (Coleman 1917), and infection of trap plants (Sreenivasaya and Rangaswami 1934). Insect species like *Coelidia indica*, *Moonia albimaculata*, *Coccosperphus tubermaculatus*, *Nezara viridula*, *Macrosiphum* sp., *Nephotettix virescens* and *Redarator bimaculatus* are probably natural vectors of sandal spike but the validity of transmission by these insects appears doubtful (see Raychaudhuri and Varma 1980, 1988).

The lace bug *Stephanitis typica*, leaf hopper *Sophonia greeni*, and plant hopper *Proutista moesta* are constantly associated with coconut palms affected with root (wilt) disease. However, MLOs have been found in the brain and salivary gland of the lace bug and not the leaf and plant hopper after acquisition access and latent periods of up to 23 days—indicating that the disease might be spread in nature by the lace bug. This has been further corroborated by a linear correlation between the number of lace bugs which colonize the palms and fresh incidence of the disease (see Solomon *et al.* 1989).

The most important vector of YTDs in India is *Hishimonus phycitidis* which efficiently transmits brinjal little leaf disease under natural conditions (Thomas and Krishnamurthy 1939), and *Orosius albicinctus* which transmits sesamum phyllody (Sahambi 1970), potato purple top roll, potato marginal flavescence, and potato witch's broom diseases (Nagaich *et al.* 1978). Both the insects are common throughout the country and produce a high incidence of various YTDs, by transmitting the MLOs from secondary hosts to the important crop plants. *Exitianus fusconervosus* transmits peach 'X' disease from peach to celery and periwinkle (Ahlawat and Chenulu 1979).

Coleman (1923) reported transmission of spike disease from natural hosts of sandal affected with similar diseases to healthy sandal through haustorial connections. This, however, has not been confirmed by Rangaswami and Griffith (1939), under better controlled conditions suggesting that Coleman's (1923) experimental plants might have been infected through a natural insect vector. Occasionally, sandal also autohaustorizes which may allow passage of MLOs from diseased to healthy plants but so far experimental evidence for such transmission is wanting (see Raychaudhuri and Varma 1988).

In vegetatively propagated crops like fruit trees, potato and sugar cane, etc., MLOs are transmitted through infected seed material. This method of MLO dissemination is very important as it is responsible for wider spread of these pathogens. In diseases like rubbery wood of apple and citrus, transmission of casual MLOs by infected budwood is the only method of disease spread.

Management of YTDs. Elimination of source(s) of infection, modification of cultural practices, chemical control of vector, chemotherapy, and host resistance have been tested to manage various YTDs.

Between 1934 and 1936, 50,000 spiked and 25,000 healthy sandal trees were uprooted to create a two-mile wide strip of land free from not only spiked trees but also other hosts affected by YTDs but it could not contain the disease (see Raychaudhuri and Varma 1988). Although, even large scale removal of diseased sandal trees is not effective in containing the spike disease, removal of YTD-affected trees is recommended as a part of integrated management of YTDs of perennial plants (Varma *et al.* 1991). In clonally propagated species the use of healthy planting material is essential as these are the major sources of primary infection and spread of diseases. Similar programmes would be effective in managing YTDs of clonally propagated species in India too. The elimination of weed hosts of MLOs which cause YTDs in annual crops should provide effective control of these diseases but it has not been tested.

For the control of sandal spike the use of hosts which impart

resistance in sandal to spike disease has been recommended (see Raychaudhuri and Varma 1988). Of particular interest is the 'complete' resistance of sandal to spike disease, when cultivated with Mysore gum planted at 10 to 20 m spacing (Sen-Sarma 1977). Similarly, the use of sweet lime and pummelo as root-stock provides resistance to infection by greening BLO in *Citrus sinensis* (Nariani *et al.* 1975). However, these observations need confirmation.

The most exciting discovery of the association of MLOs and BLOs with YTDs has been the possibility of the chemotherapeutic control of YTDs. Various antibiotics, systemic fungicides, and their combinations not only lead to recovery of treated plants under glasshouse conditions but also under field conditions. The treatment with tetracycline antibiotics like dimethylchlorotetracycline hydrochloride, and fungicides like benomyl (methyl 1-butylcarbamoil-2-benzimidazole-carbamate), singly or in combination, result in remission in symptoms which may persist for up to six months in the case of sandal affected with spike disease, or for the full growing season in the annual plants (Varma *et al.* 1975, Raychaudhuri and Varma 1988, Raychaudhuri and Rishi 1989). Tetracycline therapy is effective in the remission of symptoms caused by peach 'X' disease in *Prunus* spp. (Ahlawat *et al.* 1984).

The chemotherapy does not provide a permanent cure and the recovered parts become infected after the cessation of treatment. The treatment of perennial trees with tetracycline antibiotics is recommended because it is effective and the chemical does not contaminate the food and fodder. But chemo-therapeutic treatment, with tetracycline antibiotic of annual crops, particularly vegetables, should be used with utmost care as the intake of tetracycline antibiotics through food results in their accumulation in human or animal body making the use of these life-saving drugs ineffective, due to development of resistance in pathogenic bacteria by exposure to small doses of antibiotics.

The heat treatment is also effective in controlling YTDs. Hot water treatment of sugarcane setts at 50°C for two hours, or moist air treatment at 54°C for four hours cure the setts infected with grassy shoot MLO (Vasudeva 1956, Singh 1968, Srivastava *et al.* 1977). The hot air treatment is used in this country on a commercial scale. The potato tubers infected with potato phyllody MLO also recover by heat treatment at 50°C for 10 to 15 minutes (Khurana *et al.* 1979).

Reducing the population of vector(s) of MLO diseases is one of the most effective approaches for management of YTDs. Scanty success has been achieved in managing sesamum phyllody by using insecticides against its insect vector (Barar and Sandhu 1976). In the endemic areas of YTDs, a spraying schedule with safer insecticides like

phosalone and fenvalerate are effective. Besides, dimethoate and phosphamidon are also very useful to reduce the population of the vector (Kushwaha *et al.* 1989).

The only feasible solution to control YTDs is through the development of disease-resistant/tolerant varieties, or by the use of resistant root-stocks in vegetatively propagated crops. Much work has not been done on this aspect in India, and also in other parts of the world. Natural cross-dwarf coconuts show tolerance to coconut-(root) wilt (Rawther and Pillai 1972). Nagaich *et al.* (1978) report resistance against potato YTDs. Certain hosts like *Azadirachta indica* can provide resistance to sandal against spike disease (Raychaudhuri and Varma 1988). Further efforts are necessary to breed for resistance to YTDs, and also to utilize the latest biotechnological tools for imparting resistance in plants to specific diseases (Varma *et al.* 1991).

CONCLUDING REMARKS

The demonstration of the association of Mollicutes and BLOs with YTDs has given hope for curing at least the valuable perennial plants affected with YTD. The possibility of a cure of sandal affected with sandal spike (Raychaudhuri *et al.* 1972), one of the most serious diseases of plants in India, and citrus affected with greening (Nariani *et al.* 1975), another serious disease, by antibiotic treatment under field conditions are major break throughs in minimizing economic losses caused by such diseases.

The etiology of various YTDs in the country is not well understood. Further efforts are essential to satisfy Koch's postulates and to understand host-pathogen-vector interactions. Epidemiological information is also scanty, mainly due to the difficulties in correct diagnosis. MLOs are associated with a large number of plant species affected with YTDs. Their identification and characterization is also essential. Only then the effective management of various YTDs would be feasible. Surprisingly, *Spiroplasma* has not been detected in India so far, but it is a matter of time before these are detected.

Although none of the MLOs has so far been cultured on synthetic media, their habitat suggests that it should be possible to culture them if the nutrients and conditions for growth match the environment provided by sieve tubes. Improved technologies and sustained efforts will certainly help to culture and characterize MLOs. The methodologies for the isolation and purification should be further improved to obtain preparations of the MLOs of higher purity for use in their genome characterization and epitope typing. In the immediate future molecular diagnostic probes like monoclonal antibodies and c-DNA would be available for a large number of MLOs and BLOs which cause diseases of economic plants. These would be very useful in distinguishing

naturally-occurring variants of this important group of pathogens, and understand their epidemiology and interactions with the host for quick identification of resistant genotypes. Such developments would prove extremely valuable in devising integrated management practices for all the important YTDs and, ultimately, complete freedom from such manageable diseases.

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