

Presence of *Mycoplasma*-like bodies in the phloem of sandal affected with spike disease

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The spike disease of sandal trees (*Santalum album* L.) belongs to the so-called yellows-type diseases which are characterized by chlorosis, growth stimulation of normally dormant buds, virescence of flowers and an abnormally erect growth. As far as known from the literature the insect vectors are all Cicadellidae. Further, the causal agents of yellows-type diseases can easily be transmitted by grafting.

Until recently these pathogens were considered to be viruses. However, Doi et al. (1967) were the first to report the presence of structures resembling *Mycoplasma* (pleuropneumonia-like organisms) in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows and *Paulownia* witches' broom. Later *Mycoplasma*-like organisms have also been reported in plants affected with other yellows-type diseases such as corn stunt (Maramorosch et al., 1968), stolbur of the tomato (Cousin et al., 1968), clover phyllody (Cousin et al., 1968), apple proliferation (Gianotti et al. 1968), "Mal Azul" of the tomato (de Lourdes V. Borges and David-Ferreira, 1968) and Crimean yellows, clover dwarf and parastolbur in *Vinca rosea* (Ploaie and Maramorosch, 1969). With these findings in mind, we have carried out electron microscopical studies of thin sections of sandal plants showing symptoms of the spike disease.

Longitudinal slices, 1 mm thick, sectioned from a disc of a twig of a diseased sandal tree were immediately fixed in a solution of 1% OsO₄ in 0.1 M phosphate buffer at pH 7.2-7.4. During 2 h of fixation the material-containing test tubes were kept in ice. Thereafter, the slices were dehydrated in increasing concentrations of alcohol (50 and 70%). As no methacrylate monomer was available at the time of preparation the slices were kept in 70% alcohol for some time. Thereupon they were transferred to the methacrylate monomer via alcohol solutions of 96 and 100%. The phloem parts of the slices were carved out and embedded. The embedding was done in a pre-polymerized methacrylate mixture of butyl- and methylmethacrylate (4:1) which was polymerized in gelatin capsules at 60°C for 24 h (Pease, 1960). Ultra-thin sections were cut with glass knives on a LKB-ultramicrotome. The sections thus obtained were collected on grids covered with a formvar film and subsequently stained with uranyl acetate followed by lead-citrate (Reynolds, 1963). Thereafter, they were examined with a Siemens Elmiskop I electron microscope.

Mycoplasma-like bodies were found to occur in many of the sieve elements examined (Fig. 1 and 2). Their structure was comparable with the one described by Doi et al.

Fig. 1. Electron micrograph of a longitudinal section of a sieve tube (ST) from a sandal twig affected with spike disease. Note the accumulation of *Mycoplasma*-like bodies (B) in one of the sieve-tube members. SP = part of the sieve plate.

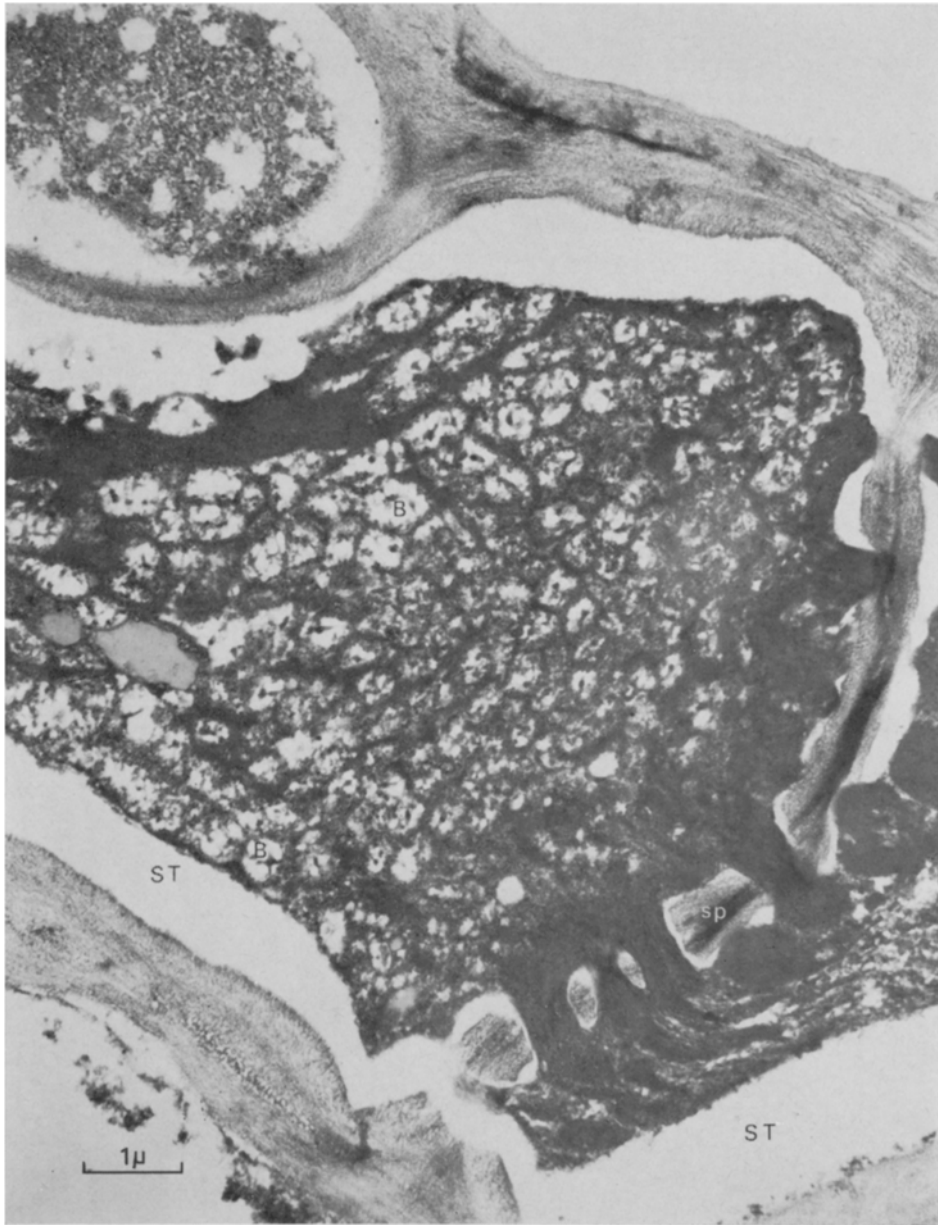


Fig. 1. Elektronenmicroscopische foto van een lengtedoorsnede door een zeefvat (ST) afkomstig van een sandeltwig aangetast door de "spike"-ziekte. Een ophoping van Mycoplasma-achtige lichaampjes (B) is zichtbaar in een van de leden van het zeefvat. SP = deel van de zeefplaat.

Fig. 2. *Mycoplasma*-like bodies in a phloem cell of a twig from a sandal tree affected with spike disease. The bigger bodies (B) show a central area with a strand (S) and a peripheral area consisting of granular material; the smaller bodies (SB) consist of a ground substance of high density.

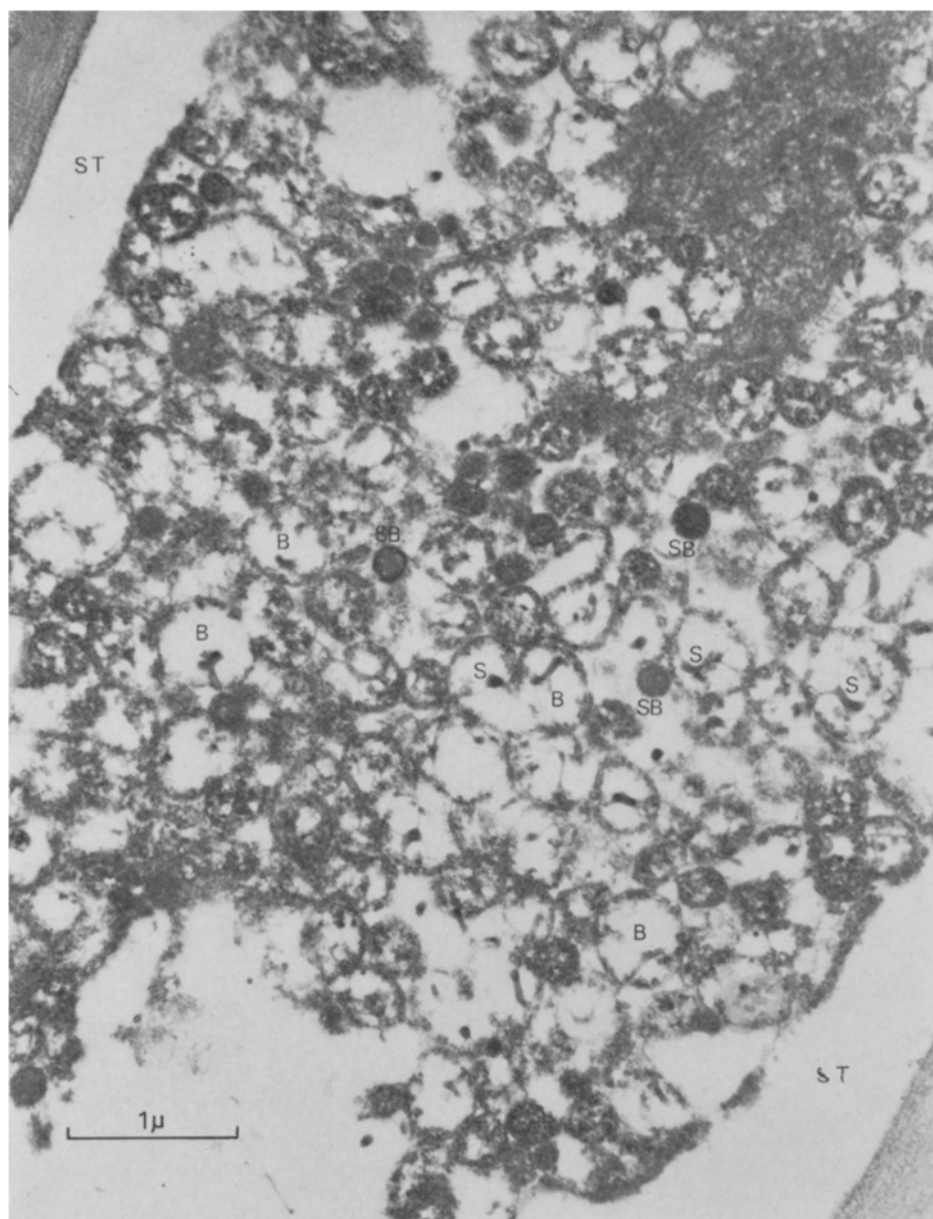


Fig. 2. *Mycoplasma*-achtige lichaampjes in een floëmccl van een twijg afkomstig van een door de "spike"-ziekte aangetaste sandelboom. De grootste lichaampjes (B) vertonen in het midden een streng (S) en aan de rand een korrelige structuur; de kleinste lichaampjes (SB) bestaan uit een optisch dichte grondmassa.

(1967). Slightly irregularly shaped, round bodies (B), ranging in size from about 300 to 600 millimicrons, with strands (S) of presumed DNA in the less electron-dense areas in the centre, and granular structures at the periphery were seen along with smaller, round bodies (SB), about 150–250 millimicrons in diameter, filled with electron-dense granular material (Fig. 2). The above-mentioned bodies could neither be demonstrated in healthy sandal material nor in parenchyma tissue of diseased leaves. Whether the *Mycoplasma*-like bodies are the causal agent of the sandal spike disease has still to be proven.

Earlier reports on the presence of inclusion bodies in parenchyma tissue (Narasimhan, 1928, 1933) and in epidermal cells (Dijkstra, 1968) of diseased sandal trees, and the present report on the localisation of *Mycoplasma*-like organisms in the phloem elements suggest that in case of sandal spike disease the inclusion bodies are not caused by *Mycoplasma*. So far there is no evidence of inclusion bodies having been caused by *Mycoplasma* in any other yellows-type disease. In sandal spike disease both *Mycoplasma* and virus might be involved.

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Samenvatting

De aanwezigheid van Mycoplasma-achtige lichaampjes in het floëem van de sandelboom aangetast door de "spike"-ziekte

De "spike"-ziekte van de sandelboom (*Santalum album* L.) behoort tot de zogenaamde "yellows-type" ziekten, die gekenmerkt zijn door chlorose, het voortijdig uitlopen van slapende knoppen, vergroening van de bloemen en een abnormaal rechtopgaande groei. Aanvankelijk werd aangenomen, dat de verwekkers van deze ziekten virussen waren. In 1967 werd echter door Doi et al. aangetoond, dat zich in het floëem van planten, die aangetast waren door vertegenwoordigers uit de groep van de "yellows-type" ziekten, *Mycoplasma*-achtige lichaampjes bevonden. Op grond van deze waarnemingen hebben we elektronenmicroscopisch onderzoek verricht aan ultra-dunne coupes van sandelplanten, die symptomen van de "spike"-ziekte vertoonden. Lengte-coupes (1 mm dik) van een schijfje van een zieke twijg werden onmiddellijk gefixeerd, ontwaterd en ingebed. De coupes werden gemaakt met behulp van een LKB-ultramicrotroom.

Mycoplasma-achtige lichaampjes bleken in een groot aantal van de onderzochte floëemelementen aanwezig te zijn (Fig. 1 en 2). Hun structuur kwam overeen met die welke door Doi et al. (1967) is beschreven. Enigszins onregelmatig gevormde, ronde lichaampjes (B), in grootte variërend van circa 300 tot 600 millimicron, met in het midden vermoedelijk uit DNA bestaande strengen (S) en een korrelige structuur aan de rand, kwamen voor naast kleinere, ronde lichaampjes (SB), met een diameter van 150–250 millimicron, die geheel gevuld waren met korrelig materiaal (Fig. 2).

De bovenbeschreven lichaampjes werden niet gevonden in coupes van gezonde sandelbomen en evenmin in het parenchym van zieke bladeren.

Met het aantonen van de aanwezigheid van *Mycoplasma*-achtige lichaampjes in zieke sandelbomen is echter nog niet bewezen, dat een dergelijk organisme inderdaad de verwekker van de sandel "spike"-ziekte is.

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