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Transmission by dodder of sandal spike disease and the accompanying mycoplasma-like organisms via Vinca rosea

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Abstract

Healthy *Vinca rosea* plants, connected by dodder strands with spike-diseased sandal trees developed witches' broom symptoms. Electron microscopy of petioles of the infected *Vinca* plants revealed the presence of numerous mycoplasma-like bodies. Fresh dodder established on the *Vinca* plants with witches' broom symptoms and trained onto healthy sandal trees, transmitted the spike disease back to the latter.

Introduction

Spike disease of sandal (*Santalum album*), an important disease in India, belongs to the so-called yellows-type diseases characterized by witches' broom symptoms. Mycoplasma-like bodies are known to occur in the phloem of affected sandal plants (Dijkstra and Ie, 1969; Hull et al., 1969; Varma et al., 1969).

The disease is known to be transmitted from diseased sandal trees to healthy ones by bud, bark and twig grafting (Coleman, 1917) and by inserting parts of fresh, diseased leaves between wood and bark of branches or stems of healthy plants (Sreenivasaya, 1930).

Although witches' broom phenomena on other plants common in sandal areas (e.g. *Stachytarpheta indica, Dodonaea viscosa, Lantana camara, Vinca rosea*) are known to occur, experiments in which parts of diseased sandal trees were grafted onto the above-mentioned plants failed to show any transmission (Dijkstra, unpublished).

Sandal parasitises the roots of many plant species. Mutual haustorial connections between roots of the sandal trees are also possible. Coleman (1923) suggested that the natural transmission of spike disease of sandal might take place only through haustorial contacts. In his experiments, however, the possibility of transmission by insects had not been excluded. Healthy sandal trees parasitising plant species in the undergrowth which showed witches' broom symptoms remained healthy (Coleman, 1923).

Insect transmission has been reported (Anonymous, 1933; Rangaswami and Sreenivasaya, 1935; Rangaswami Iyengar and Griffith, 1939) but some doubts still exist as to the identity of the vector(s). Rangaswami and Griffith (1941) reported that the leafhopper *Jassus indicus* was a vector but indicated the need for confirmation, which, to our knowledge, has not been done.

Narasimhan (1954) described experiments in which *Cassytha* plants (a phanerogamous parasite belonging to the Lauraceae) grown on spike-diseased sandal trees

were trained onto healthy sandal plants. According to him, the results were 'somewhat promising' but needed confirmation.

Summarising, we may say that spike has been transmitted experimentally from sandal to sandal only by grafting, though there are strong indications that insects are involved in spreading the disease under natural conditions. We have tried to find whether the disease can also be transmitted by other means and whether other plant species can be infected by the pathogen as well.

Materials and methods

The sandal trees were grown from seed and maintained in big pots in the glasshouse. In the same pots *Pongamia glabra*, grown from seed, was planted on which the sandal could haustorise.

Shoots of dodder (*Cuscuta subinclusa*), grown from seed and maintained on two healthy *Vinca rosea* 'Bright Eye' plants (periwinkle) were attached to 'spiked' shoots (i.e. shoots with witches' broom symptoms) of two $3\frac{1}{2}$ year-old sandal trees which had been grafted with diseased sandal material 9 months earlier. The sandal trees had shown typical spike symptoms about 6 months after grafting. The dodder formed haustoria on the 'spiked' shoots and branches. Five weeks after attaching the dodder to the diseased sandal trees the two original *Vinca* plants were replaced by three healthy ones onto which dodder strands from the diseased trees were trained.

Fig. 1. *Vinca rosea*, infected with sandal spike disease when young (left), and healthy (right).

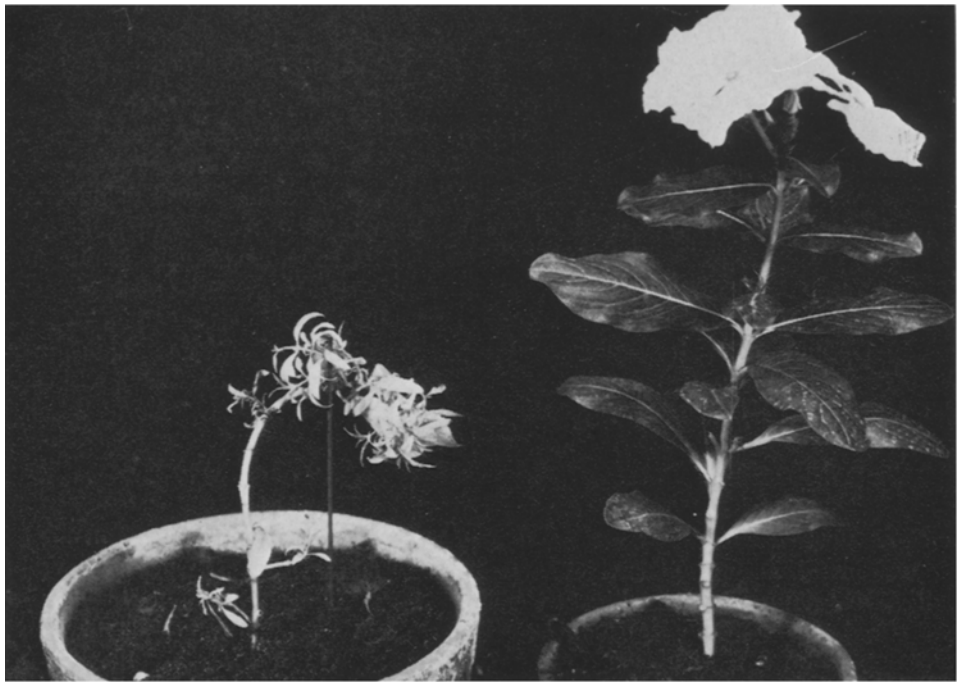


Fig. 1. Vinca rosea, in een jong stadium geïnfecteerd met de 'spike'-ziekte van sandelboom (links) en gezond (rechts).

Fig. 2. *Vinca rosea*, infected with sandal spike disease when full-grown. A, whole plant; B, detail of an apical rosette.

Fig. 2. Vinca rosea, in volwassen stadium geïnfecteerd met de 'spike'-ziekte van sandelboom. A, hele plant; B, detail van een apicale rozet.

These *Vinca* plants were removed 12 weeks later. As controls dodder was maintained on healthy *Vinca rosea* and sandal plants.

For electron microscopy samples of ca. 2 mm^2 were taken from yellow, narrow leaves of *Vinca*. These samples were fixed for 1 h in 4.0% glutaraldehyde in 0.1 M phosphate buffer, pH 6.8. Afterwards the tissues were washed twice in phosphate buffer 0.1 M, pH 6.8, then fixed for 1 h in 1% aqueous osmium tetroxide, washed three times for 10 minutes each in water and dehydrated in increasing concentrations of ethanol (50, 70, 96, and 100%). After ethanol dehydration, tissue samples were given two changes of propylene oxide and embedded in Epon-Araldite (Mollenhauer, 1964). For sectioning an LKB ultramicrotome III was used. Sections were stained with uranyl acetate and lead citrate (Reynolds, 1963), and were examined with a Siemens Elmiskop 101 electron microscope at 80 kV.

Results and discussion

Five to 10 weeks after strands of the dodder on the first two healthy *Vinca* plants had been attached to the 'spiked' sandal trees, both plants developed vein clearing and interveinal chlorosis of the leaves, ceased to flower and one developed phyllody of flowers. Later, the newly-formed leaves were narrow, small and yellowish-green, and the plants showed stunted and bushy growth with sprouting of normally dormant buds (Fig. 1). After about 16 to 20 weeks the plants wilted and finally died.

In the second series, 2 out of the 3 healthy *Vinca* plants, on which dodder strands

from 'spiked' sandal trees had been trained, showed leaf dropping and witches' broom phenomena, such as phyllody of flowers and transformation of the latter into apical rosettes, and some yellowing of leaves (Fig. 2). As these plants were older, bigger and in full bloom when they were put into contact with the dodder on infected sandal trees, they remained less stunted and yellow than those of the first series of *Vinca* plants which were much younger when infected. Although the control *Vinca* plants on which dodder was maintained for the same length of time showed deterioration (yellowing and dropping of leaves), typical witches' broom symptoms such as phyllody of flowers, sprouting of normally dormant buds and leaf narrowing were never observed.

To prove that the disease in the yellowed *Vinca* plants was the same as that in the original 'spiked' sandal tree, the following was done. Fresh dodder was established on 3 of the above 4 *Vinca* plants with witches' broom symptoms. From them strands of dodder were trained onto 2 healthy sandal trees and the connections between the diseased *Vinca* plants and the sandal trees were maintained for 12 weeks. After about 16 weeks one of the trees showed typical spike symptoms (Fig. 3). The 3 control trees, on which dodder from healthy *Vinca* had been trained, never showed any symptoms of deterioration or witches' broom.

Electron microscopy of petioles of yellowish leaves of one of the *Vinca* plants with witches' broom symptoms revealed numerous mycoplasma-like bodies in the phloem. Fig. 4 shows the distribution of mycoplasma-like bodies over the phloem elements and Fig. 5 a detail of a sieve tube full of mycoplasma- like bodies at higher magnifi-

Fig. 3. Twigs of a sandal tree, infected with spike disease (left), and healthy (right).

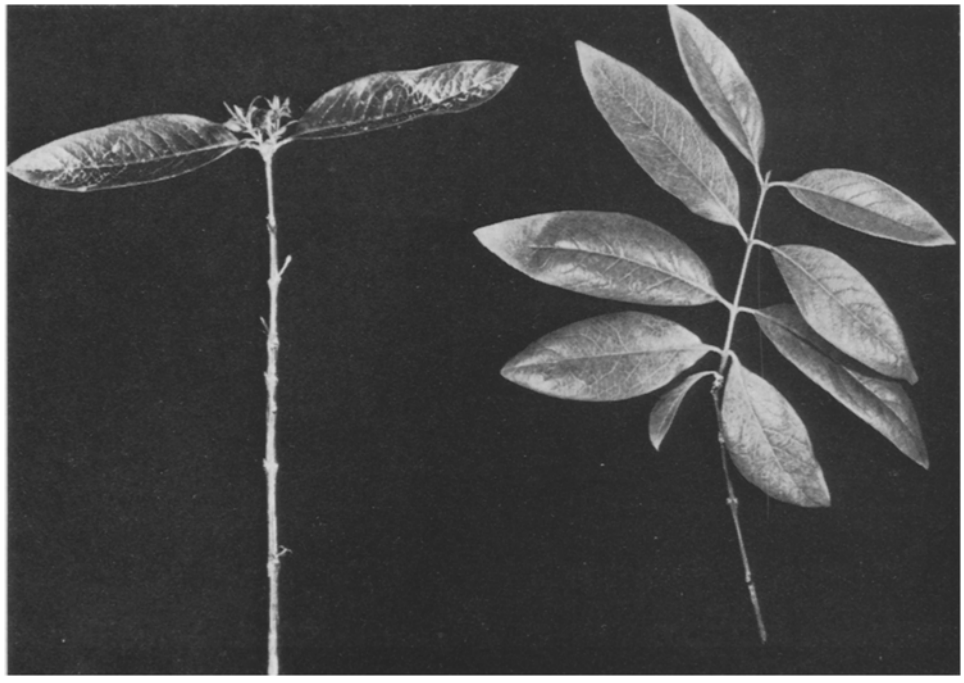
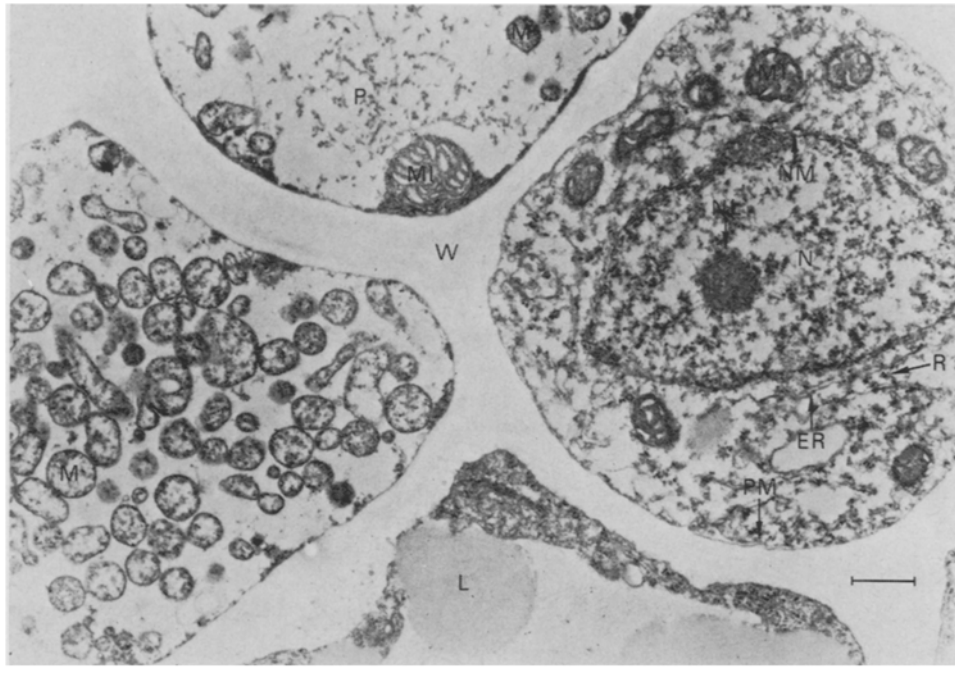


Fig. 3. Takjes van een sandelboom, geïnfecteerd met de 'spike'-ziekte (links) en gezond (rechts).

Fig. 4 (top) and 5 (below). Mycoplasma-like bodies in transverse sections of the phloem of *Vinca rosea* infected with sandal spike disease.
M, mycoplasma-like body; MI, mitochrondrion; N, nucleus; NL, nucleolus; NM, nuclear membrane; ER, endoplasmic reticulum; P, P-protein (phloem protein); R, ribosomes; W, cell wall; L, lipid body; PM, plasmalemma. Bar = 500 nm.



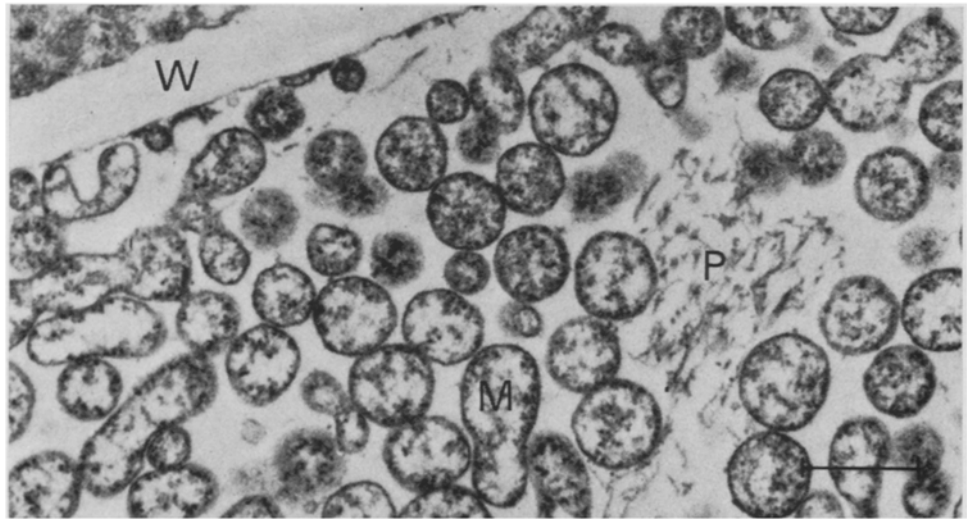


Fig. 4 (boven) en 5 (beneden). Mycoplasma-achtige lichaampjes in dwarscoupes van het floëem van Vinca rosea, geïnfecteerd met de 'spike'-ziekte van de sandelboom.

cation. These structures were comparable to those in 'spiked' sandal leaves (Dijkstra and Ie, 1969). Figs. 4 and 5 show that the majority were spherical with diameters varying from 100–200 nm for the elementary bodies to 250–550 nm for the other spherical particles. Some were ovoid, elongated or showed budding; their length varied from 250 to 1100 nm.

These results prove that the pathogen(s) from 'spiked' sandal can be transmitted by dodder to *Vinca rosea* and back to sandal. The mycoplasma-like bodies known to be present in 'spiked' sandal could also be demonstrated in the infected *Vinca* plants. Whether the mycoplasma-like organism is the only pathogen responsible for the spike disease in sandal and *Vinca rosea* could only be proved conclusively by culture of the micro-organism on cell-free media and subsequent introduction of suspensions of subcultures into healthy sandal or *Vinca* plants resulting in characteristic symptoms.

Samenvatting

Overdracht van de 'spike'-ziekte van de sandelboom en de bijbehorende mycoplasma-achtige organismen door warkruid via Vinca rosea

De 'spike'-ziekte van de sandelboom (*Santalum album*) behoort tot de heksenbezemziekten. Zoals eerder werd aangetoond bevatten door deze ziekte aangetaste sandelbomen mycoplasma-achtige lichaampjes in het floëem van takken en bladeren.

Daar alleen met zekerheid bekend is dat de ziekte door enting op gezonde sandelbomen kan worden overgebracht, is nagegaan of met behulp van warkruid (*Cuscuta subinclusa*) andere plantesoorten konden worden geïnfecteerd.

Vijf tot tien weken nadat zich op gezonde planten van *Vinca rosea* uitlopers van het warkruid, afkomstig van 'spike'-zieke sandelbomen hadden gehecht, hielden de *Vinca*-planten op met bloeien en vertoonden de bladeren nerfglazigheid en tussennervige chlorose; op sommige planten werd eveneens fyllodie waargenomen. Enige tijd later ontwikkelden zich aan scheuten van uitgelopen okselknoppen kleine, smalle, geelgroene blaadjes (Fig. 1). Na ongeveer 16–20 weken verwelkten de *Vinca*-planten en stierven af. Werden oudere planten geïnfecteerd dan bestonden de heksenbezemsymptomen hoofdzakelijk uit fyllodie, apicale rozetten en lichte bladvergeling (Fig. 2).

Door opnieuw warkruid te laten groeien op de ziek geworden *Vinca*-planten en uitlopers van de parasiet te leiden naar gezonde sandelbomen verschenen ongeveer 16 weken nadat het warkruid zich op laatstgenoemde had gehecht typische 'spike'-symptomen op een van de twee bomen (Fig. 3).

Elektronenmicroscopisch onderzoek van bladsteeltjes van vergeelde *Vinca*-blaadjes gaf een groot aantal mycoplasma-achtige lichaampjes van verschillende vorm en afmeting te zien (Fig. 4 en 5).

Hiermee is aangetoond, dat de 'spike'-ziekte van de sandelboom door warkruid kan worden overgebracht op *Vinca rosea* en weer terug op sandel en dat ook in *Vinca* de symptomen samengaan met het voorkomen van mycoplasma-achtige lichaampjes.

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