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Anatomical aspects of sandal plants affected with spike disease

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Abstract

Sections of young leaves with typical "spike" symptoms did not show any differentiation into palisade and spongy parenchyma tissue. Moreover, the regular structure of the vascular bundles was sometimes slightly disturbed and often necrotic cells occurred both in the phloem and in the cambial zone.

Cross sections of diseased stems showed that the organisation of the phloem, medullary rays and sometimes also of the cambial layers was disturbed. Besides, numerous groups of necrotic cells could be observed in the phloem and, less frequently, in the cambial layers.

Longitudinal sections of diseased stems stained with methyl green–pyronin showed an accumulation of blue material. As even at a magnification of × 1500 no differentiation of the blue material could be obtained, the latter might consist of accumulated DNA from *Mycoplasma*-like organisms having lost their original structure as a result of the fixation and embedding techniques used.

Introduction

Spike disease is a serious disease of the sandal tree (*Santalum album*) in South India. The first published record of the disase is contained in the report of the Forest Administration in Coorg during the year 1898–99.

Within about 2 years after appearance of the first macroscopically visible symptoms, consisting of phyllody of flowers, prominent narrowing of leaves which stand out stiffly from the branch and other witches' broom phenomena, the affected tree dies.

Coleman (1917) demonstrated that the causal agent could be transmitted by grafting. Later, it was shown that it could also be transmitted by leafhoppers (Rangaswami and Sreenivasaya, 1935). Recently, the presence of *Mycoplasma*-like organisms in spike-diseased sandal has been reported (Dijkstra and Ie, 1969; Varma et al., 1969).

The anatomy of diseased sandal plants has been studied by Barber (1903), Butler (1903), Coleman (1917), Narasimhan (1933), and Dijkstra (1968). The former three authors gave a detailed description of various abnormalities in spike-affected trees; the latter two authors reported the presence of inclusion bodies in leaf parenchyma tissue and petioles, and in leaf epidermis, respectively.

In order to gain more insight into the pathogenesis a comparative anatomical study was carried out on leaves and stems of healthy and diseased sandal trees.

Materials and methods

The diseased material was obtained from Chamundi Hill, Mysore (India) which is a naturally infected area, and from our glasshouse at Wageningen where 2-year-old sandal seedlings had been grafted with diseased material from Chamundi Hill in

April 1969; the latter plants showed symptoms about 3 months after grafting. Likewise, two batches of healthy material were used, one from the Chamundi Hill (collected from healthy-looking trees) and the other from 2-year-old seedlings grown in our glasshouse.

Leaves and stems were fixed either in Carnoy's fluid consisting of 3 parts of 96% alcohol and 1 part of glacial acetic acid, or in FAA (5 parts of 40% formaldehyde, 90 parts of 50% alcohol, and 5 parts of glacial acetic acid). The material was embedded in paraplast, and 8 or 12 μ thick sections were made with a microtome. Two stains, methyl green–pyronin and 1% toluidine blue, were used. Sections of fixed stems were transferred to slides and brought down to distilled water by the standard method. The sections were stained for 10 min, rinsed in distilled water, drained and blotted carefully. After dehydration in alcohol they were mounted in xylene-dammar. In some cases leaves were stained with Heidenhain's iron-alum haematoxylin.

Results and discussion

Leaf. A cross section of a healthy, young sandal leaf consists of a cuticle, epidermis, hypodermis, two layers of palisade parenchyma, a varying number of layers of spongy parenchyma with calcium oxalate crystals, vascular bundles (the bigger ones with a prominent bundle-sheath extension), epidermis and cuticle (Fig. 1A). Sections of young leaves with typical "spike" symptoms showed the following abnormalities (Fig. 1B). There was no differentiation into palisade and spongy parenchyma tissue. The regular structure of the vascular bundles was sometimes slightly disturbed especially regarding the course of the medullary rays. Often necrotic cells occurred in the phloem and in the cambial zone.

Fig. 1. A: Transverse section of healthy sandal leaf, stained with toluidine blue. The mesophyll is differentiated into palisade (P) and spongy parenchyma (S).
B: Transverse section of diseased sandal leaf, stained with toluidine blue. The mesophyll is undifferentiated.

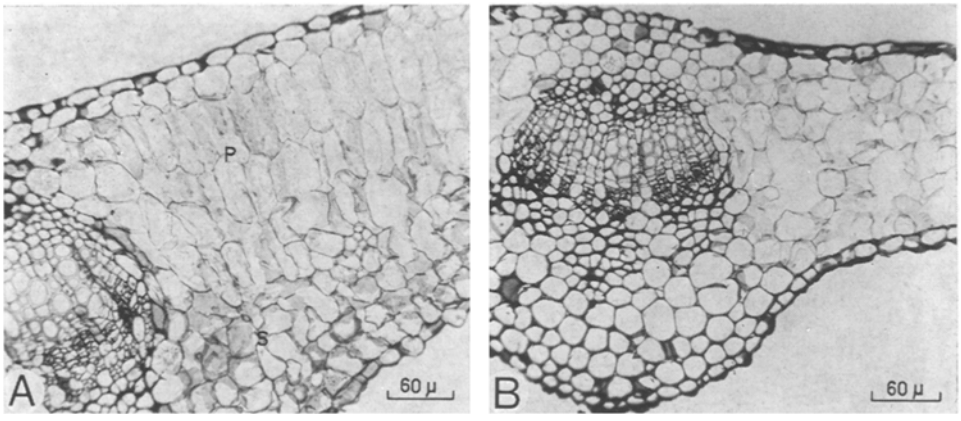

Fig. 1. A: Dwarsdoorsnede door een gezond sandelblad, gekleurd met toluïdineblauw. Het mesofyl is gedifferentieerd in palissade- (P) en sponsparenchym (S).
B: Dwarsdoorsnede door een ziek sandelblad, gekleurd met toluïdineblauw. Het mesofyl is ongedifferentieerd.

Fig. 2. A: Transverse section of healthy sandal stem, stained with toluidine blue. Obliterated cells (O) can be seen in the phloem.
B: Transverse section of diseased sandal stem, stained with toluidine blue.
Besides normally obliterated cells (O) groups of necrotic cells (N) occur in the phloem and in the cambial layers. The course of the medullary rays (MR) is disturbed.

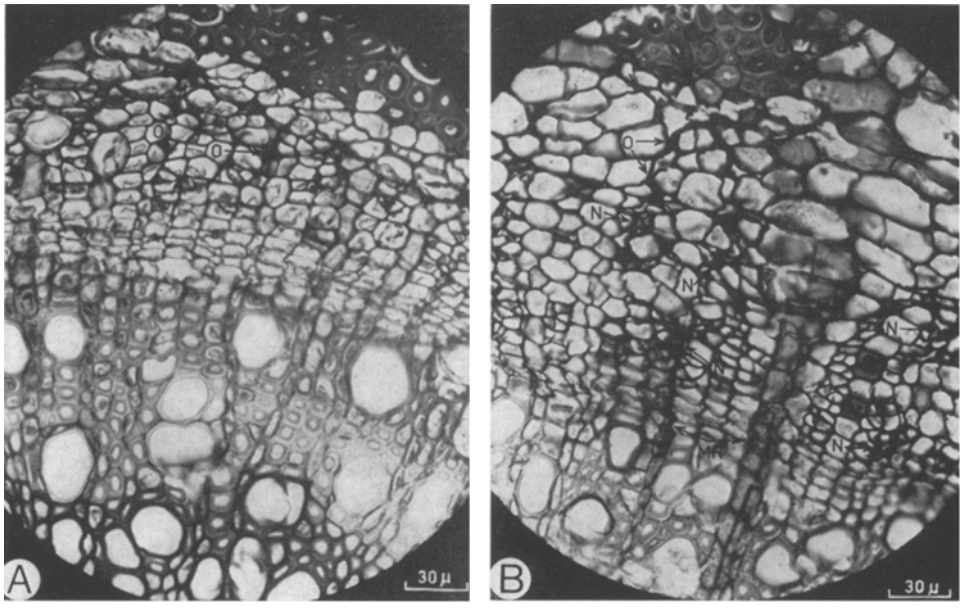

Fig. 2. A: Dwarsdoorsnede door een stengel van een gezonde sandelplant, gekleurd met toluïdineblauw. In het floëem zijn gedesintegreerde cellen zichtbaar.
B: Dwarsdoorsnede door een stengel van een zieke sandelplant, gekleurd met toluïdineblauw. Behalve de gedesintegreerde cellen in het floëem (O) treden groepjes necrotische cellen (N) op in het floëem en de cambiale lagen. De loop van de mergstralen (MR) is verstoord.

In contrast to what has been reported by Narasimhan (1933) no inclusion bodies could be found in the parenchyma cells of affected leaves. In most of these cells the nuclei looked normal but in some the nucleoli were found to be bigger than those in cells of healthy leaves. The discrepancy between Narasimhan's results and those of the present authors might be attributed to the fact that in the latter studies young "spiked" leaves were used whereas in the former very old ones. According to Narasimhan "...it is in such cells that the bodies stand out prominently."

No inclusion bodies were visible in the epidermal cells either. This is not surprising as the incidence of the inclusion bodies in this tissue is low with 1 % of the epidermal cells containing such bodies (Dijkstra, 1968).

Stem. A cross section of a healthy sandal stem (1.3 mm in diameter) showed a cuticle, epidermis, cortex with calcium oxalate crystals, phloem with bundles of phloem fibers, cambial layers, xylem (secondary and primary), medullary rays, and pith in which sometimes calcium oxalate crystals were present (Fig. 2A). In a diseased stem of about the same size (1.6 mm in diameter) the organisation of the phloem, medullary

rays and sometimes also of the cambial layers was disturbed. Although the presence of some obliterated cells is a normal phenomenon in phloem of healthy stems, the occurrence of numerous groups of necrotic cells could only be observed in the phloem and, less frequently, in the cambial layers of diseased stems (Fig. 2B).

Longitudinal sections of diseased stems stained with methyl green–pyronin showed an accumulation of the stain in the sieve tubes, particularly in the regions near the sieve plates. At a magnification of × 1500 the densily stained regions proved to contain granular material which resembled very much the chromophilous substances described by Borges and David-Ferreira (1968) in sieve tubes of tomato stems affected with 'Mal Azul'. The latter authors suggested that the granules might be *Mycoplasma*-like organisms which they could demonstrate electron microscopically in ultrathin sections of this tissue. However, in our investigations these granules were also present in sections of healthy stems so that no conclusion can be drawn about a possible presence of *Mycoplasma*-like organisms which previously have been shown to occur in ultrathin sections of sieve tubes of stems affected with spike disease (Dijkstra and Ie, 1969). The only difference between longitudinal sections of healthy and diseased stems was that in the latter a part of the densily stained regions near the sieve plates was blue and the rest of the sieve tube contents red, whereas in the former only a red colour was observed. No further differentiation of the blue material could be obtained at a magnification of × 1500. It is of course conceivable that the blue colour must be attributed to an accumulation of DNA from *Mycoplasma*-like organisms which might have lost their original structure due to the fixation and embedding techniques used.

When we compare the above findings with those reported by Barber (1903), Butler (1903) and Coleman (1917), it is remarkable that in many respects there are serious discrepancies. Butler observed that in a diseased leaf "...a well-marked palisade parenchyma is formed which is absent in the healthy leaf." This is quite the contrary of what we have found. Coleman's observations do not agree with those of Butler. The former author found that with the ageing of the leaves mesophyll cells elongated at right angles to the surfaces of the leaves, and this elongation was even more marked in healthy than in diseased leaves. Coleman compared young and old healthy leaves with young and old diseased leaves and he attributed Butler's observation to the fact that the latter might have compared the structure of young, healthy leaves with mature "spiked" ones. Our results obtained with young healthy and diseased leaves are also not in agreement with those of Coleman.

With regard to alterations in the stems the only important feature mentioned by Barber, Butler and Coleman was the excessive deposition of starch in the pith, medullary rays, and xylem and phloem parenchyma. As the starch content of healthy trees depends on the time of the year (accumulation of starch in the twigs when the leaves are mature) comparisons between healthy and diseased trees can only be made in the growing season, as has already been pointed out by Coleman. No other striking differences in the anatomy of healthy and diseased sandal stems have been observed by the above-mentioned authors.

The conclusion must be drawn that no valid comparison between results of these different studies is possible in the absence of exact data with regard to age of the sandal tree, stage of infection, size of the leaves used and their nature, whether young, green and turgid or old, yellowish and shrivelled. In order to arrive at serious conclusions, a systematic study has to be carried out of all the different stages of infection

throughout the years, beginning with the moment a tree shows the first macroscopically visible symptoms to the last stage of infection at which the spike-affected tree drops its remaining leaves.

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Samenvatting

Anatomisch onderzoek van sandelplanten, aangetast door de "spike" ziekte

Voor kleuring van de 8 of 12 μ dikke microtoomcoupes van stengels en bladeren werden meestal methylgroen–pyronine en toluïdineblauw gebruikt; enkele bladcoupes werden ook met Heidenhain's hematoxyline gekleurd.

Dwarsdoorsneden van jonge, gezonde sandelbladeren (Fig. 1A) en jonge, zieke bladeren (Fig. 1B) werden met elkaar vergeleken. Opmerkelijk was, dat in het laatste geval de differentiatie tussen palissade- en sponsparenchym was verdwenen. Verder was de regelmatige opbouw van de vaatbundels enigszins verstoord. Vaak werden in floëem en cambiale lagen necrotische cellen waargenomen.

Bij vergelijking tussen gezonde stengels (Fig. 2A) en zieke (Fig. 2B) bleek in de zieke stengels de organisatie van floëem, mergstralen en soms ook de cambiale lagen verstoord te zijn. Voorts waren in deze weefsels groepjes necrotische cellen zichtbaar.

Lengtedoorsneden van zieke stengels, die gekleurd waren met methylgroen–pyronine, vertoonden sterk gekleurde gedeelten in de zeefvaten, vooral in de buurt van de zeefplaten. Bij een vergroting van 1500 × werden zowel bij gezonde als zieke stengels in deze gebieden korrelige structuren gevonden. Het enige verschil was, dat bij de zieke een gedeelte van deze sterk gekleurde gebieden een blauwe kleur vertoonde en voor de rest rood was, terwijl bij het gezonde materiaal alleen de rode kleur aanwezig was. Dit zou kunnen wijzen op een ophoping van DNA in het floëem van zieke stengels.

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