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An anomalous form of mycoplasma-like bodies in periwinkle infected with the sandal spike agent

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Abstract

An anomalous form of mycoplasma-like bodies was found in 'necrotic' cells in the sieve elements of periwinkle stem after infection with the sandal spike disease agent. These bodies, 50–160 nm in diameter, were strongly osmiophilic and bounded by a unit membrane. It is suggested that these anomalous bodies represent a naturally degenerated form of the mycoplasma-like bodies.

Introduction

Since the recent discovery of mycoplasma-like bodies (hereafter referred to as mycoplasmas) associated with 'yellows' diseases of plants (Doi et al., 1967), investigations in various laboratories have already revealed the association of such bodies with more than 50 plant diseases (Maramorosch et al., 1970; Davis and Whitcomb, 1971).

In the majority of the diseases investigated, the bodies were found in the phloem elements, mostly in sieve tubes and sometimes in phloem parenchyma cells. In some cases their abundance in other parenchymatous cells has been reported (Hampton et al., 1969; Cousin et al., 1970).

The life cycle of mycoplasmas is not yet understood, though for certain animal-infecting mycoplasma species speculations have been presented (Boatman and Kenny, 1970; Smith, 1971). Similar attempts have been made recently on the basis of morphological studies on thin sections of plant-infecting mycoplasmas in situ (Sinha and Paliwal, 1970; Worley, 1970).

Very little is known about morphological degeneration of mycoplasmas in infected plants (Hirumi and Maramorosch, 1972). In this report, we present the evidence for the occurrence of a naturally degenerating form of mycoplasmas in certain sieve elements of a periwinkle plant affected with the sandal spike agent.

Materials and methods

The sandal spike agent was introduced to potted seedlings of periwinkle (*Vinca rosea* 'Bright Eyes') by means of dodder (*Cuscuta subinclusa*) in an insect proof greenhouse, as described previously (Dijkstra and Lee, 1972).

Small portions of stems, about 5–8 mm from the tip, were excised from plants showing witches' broom symptoms after about 8 weeks of contact between a spike-diseased sandal tree (*Santalum album*) and periwinkle plants. Comparable portions

were sampled from healthy control plants. The excised tissues of about 2 mm^2 were fixed for 1 h in 3.0% glutaraldehyde in 0.025 M phosphate buffer, pH 7.4. The tissues were then washed twice in the same buffer, postfixed for 1 h in 1% osmium tetroxide, washed three times for 20 minutes each in the buffer, and kept in 0.5% uranyl acetate in distilled water at 4 °C overnight. The tissue samples were dehydrated in a graded ethyl alcohol series, and given two changes of propylene oxide. The materials embedded in Epon-Araldite (Mollenhauer, 1964) were sectioned with an LKB ultramicrotome III, double-stained with uranyl acetate and lead citrate (Reynolds, 1963). The sections were observed with a Siemens Elmiskop 101 electron microscope at 60–80 kV.

Results

The mycoplasmas of the sandal spike disease, like those found with other 'yellows' diseases of plants, showed an extremely simple structure (Fig. 1). They had three essential organelles: the unit membrane, 8–10 nm thick; the ribosomes of bacterial type, 12–13 nm in diameter; and the nucleoid (DNA strand) devoid of membrane.

Three main types of pleomorphic bodies were observed, viz. spherical bodies, 160–280 nm in diameter, large bodies, 400–800 nm in diameter, and bodies of 50–160 nm

Fig. 1. A section of periwinkle phloem cells showing mycoplasma. PD = plasmodesma, W = cell wall.

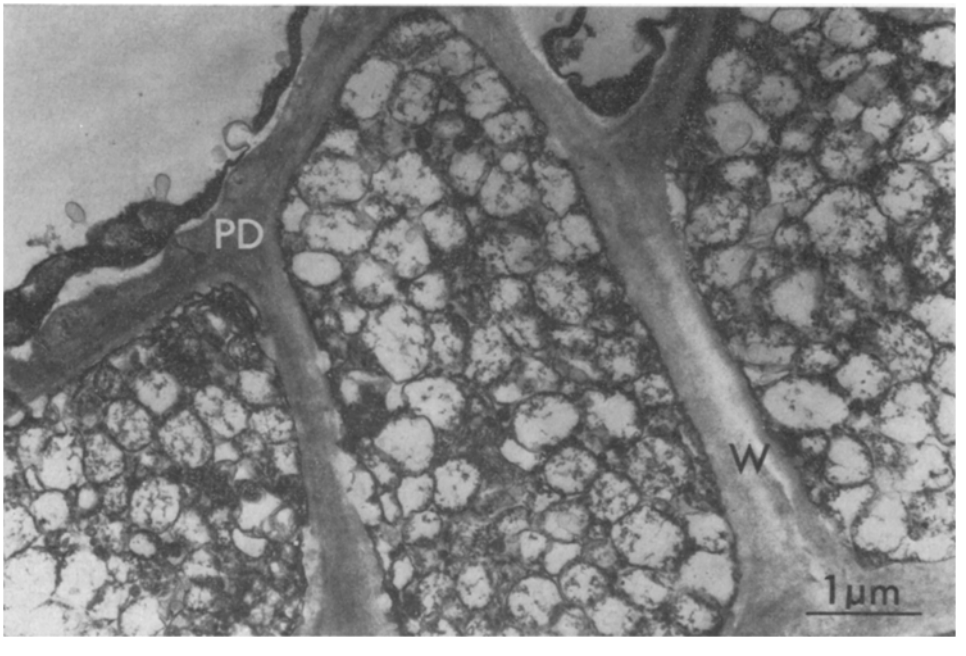

Fig. 1. Mycoplasma in een dwarscoupe van floëemcellen van Vinca rosea. PD = plasmodesma, W = celwand.

Fig. 2. A: Two types of pleomorphic mycoplasma bodies. The large bodies (arrows) show irregular distribution of the ribosomes. B: Swelling of large pleomorphic bodies. Note the budding-like projection of a large body (an arrow). The disruption of membrane is seen in swollen bodies. Some of the bodies are devoid of cytoplasmic content and others are morphologically normal.

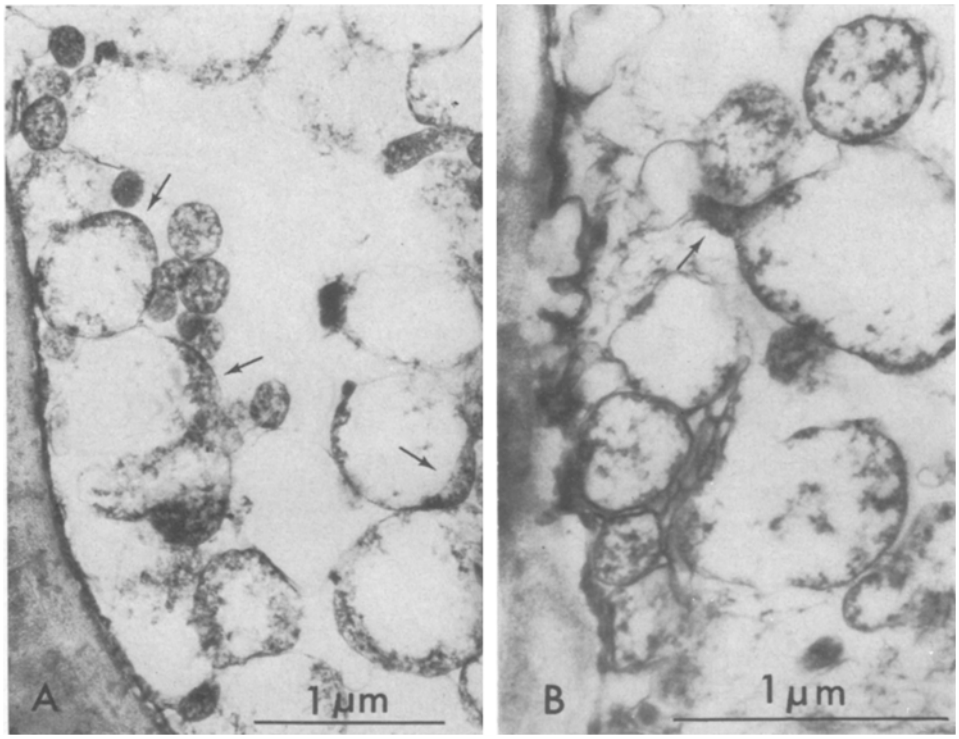

Fig. 2. A: Twee typen pleomorfe mycoplasmalichaampjes. De grote lichaampjes (pijlen) vertonen een onregelmatige verdeling der ribosomen. B: Gezwollen grote, pleomorfe lichaampjes. Let op de knopachtige uitstulping (pijl). Van verschillende gezwollen lichaampjes is de membraan gescheurd. Sommige lichaampjes hebben geen cytoplasma, andere zijn morfologisch normaal.

in diameter, strongly osmiophilic with contents condensed to various degrees (Fig. 3).

In the bodies of the first type, the ribosomes were more or less evenly distributed in the cytoplasm and no nucleoid was apparent, whereas in the cells of the second type, the nucleoid was seen in the central region. This central region, however, became electron transparent in some large bodies. The swelling of the large bodies was frequently associated with the uneven distribution of ribosomes near the periphery of the bodies (Fig. 2A and B). Indications of rupture, budding-like projections (Fig. 2B), and invagination of the cell membrane (Fig. 5) were also noted.

Most striking were the strongly osmiophilic bodies in degenerated sieve cells (Fig. 3–8). In such cells apparent remnants of both degenerating host and mycoplasmas were intermixed. The strongly osmiophilic bodies were bounded by a unit membrane, but their cytoplasmic content was without clearly visible structure due to apparent condensation (Fig. 3 and 4). The bodies were often disrupted and the membrane as

Fig. 3. Association of host plasmalemma with an anomalous form of mycoplasma bodies. DM = degenerated material, L = lipid body, PL = plasmalemma, W = cell wall.

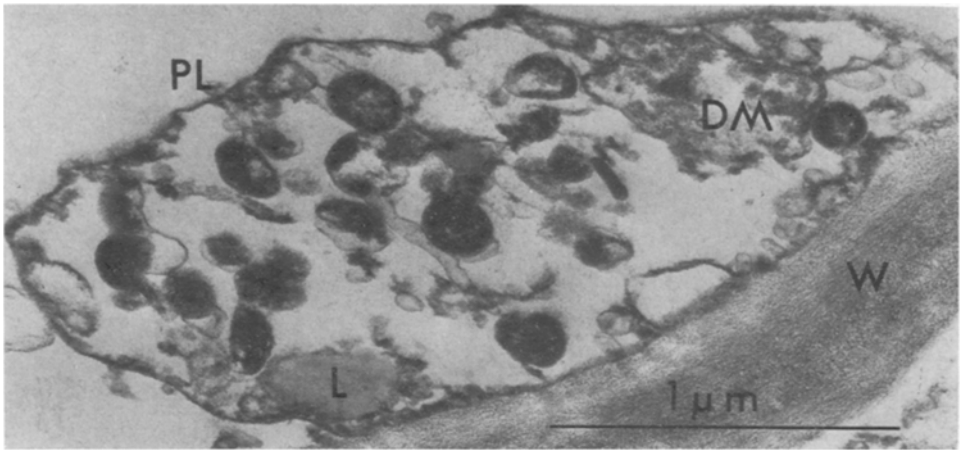

Fig. 3 Verbinding tussen het plasmalemma van de waardplant en een abnormale vorm van mycoplasmalichaampjes. DM = gedegenereerd materiaal, L = vetlichaam, PL = plasmalemma, W = celwand.

Fig. 4. Strongly osmiophilic, anomalous bodies at a high magnification. The bodies are bounded by a unit membrane (arrows) and their cytoplasm is much condensed.

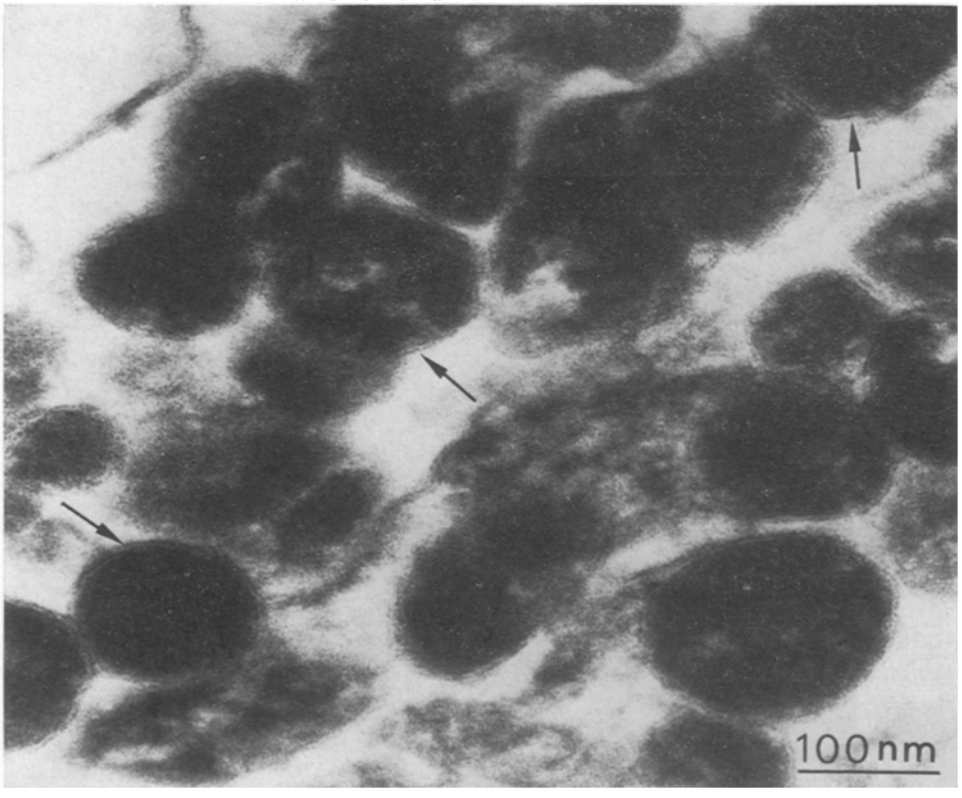

Fig. 4. Sterk osmiofiele, abnormale lichaampjes bij grote vergroting. De lichaampjes zijn omgeven door een eenheidsmembraan (pijlen) en hun cytoplasma is zeer compact.

Fig. 5. The independent occurrence of normal and abnormal forms of mycoplasma bodies in separate phloem cells. Note invagination of mycoplasma membrane (arrows) and irregular thickening of the secondary wall due to callose deposition. AF = abnormal form, CA = callose, M = mitochondrion, NF = normal form, W = cell wall.

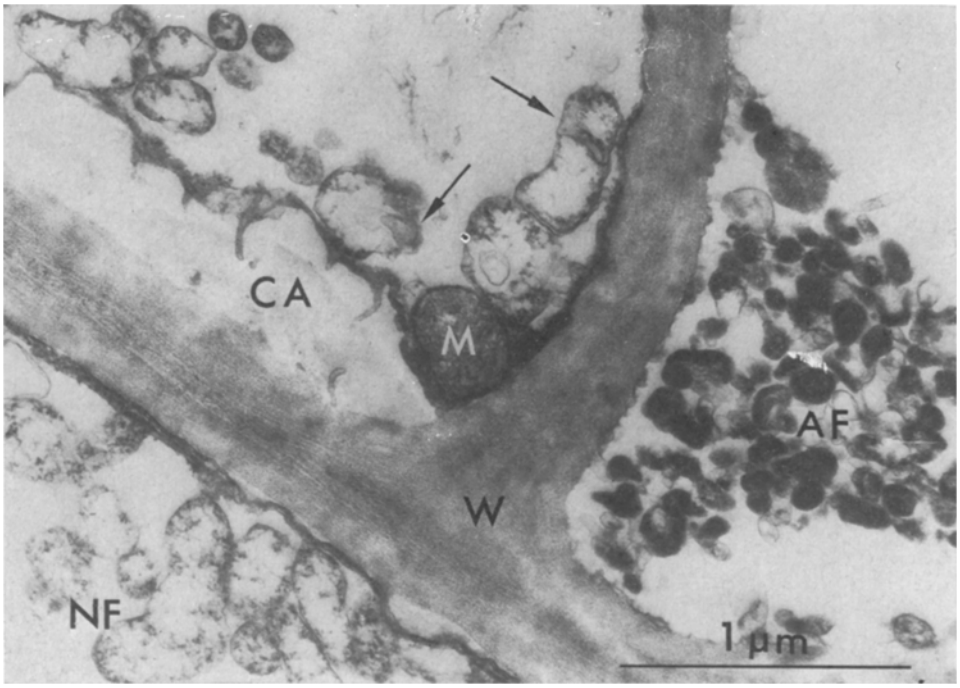

Fig. 5. Onafhankelijk voorkomen van normale en abnormale vormen van mycoplasmalichaampjes in afzonderlijke floëemcellen. Let op instulping van de mycoplasmamembraan (pijlen) en de onregelmatige verdikking van de secundaire celwand als gevolg van calloseafzetting. AF = abnormale vorm, CA = callose, M = mitochondrium, NF = normale vorm, W = celwand.

well as cytoplasmic materials resulting from the bodies were seen dispersed (Fig. 4 and 5).

This anomalous form varied in size and shape and occurred only in certain parts of affected phloem (Fig. 6–8). Even when cells were filled with such strongly osmiophilic material, the bodies still had a clearly visible membrane (Fig. 6 and 8). The sieve cells containing these anomalous bodies were in the course of obliteration (Fig. 6 and 7).

The first two mycoplasma types mentioned were found in much younger phloem cells of the same tissue (Fig. 7). Abnormal amounts and irregular distribution of callose were observed on the secondary walls of the phloem cells in which many mycoplasma bodies of the normal types were found (Fig. 5 and 7).

Discussion

In this investigation, normal and abnormal populations of mycoplasmas were found in the same thin sections of phloem tissue. The two types of normal bodies were similar

Fig. 6. Sieve cells packed with an anomalous form of mycoplasma bodies. Arrows point to the unit membranes that bound the bodies or their remnants. Note tubule-like structures of different sizes and shapes. PD = plasmodesma, W = cell wall.

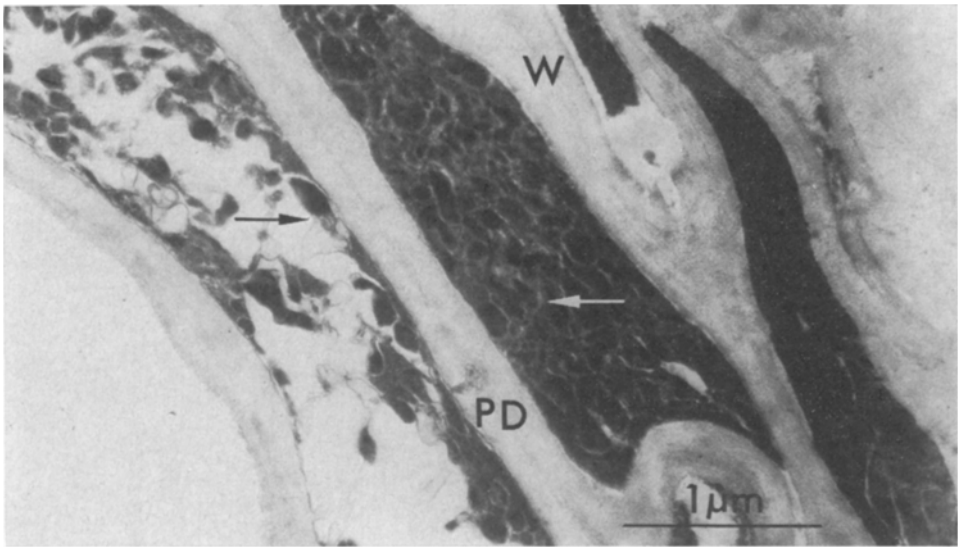

Fig. 6. Zeefcellen, vol met een abnormale vorm van mycoplasmalichaampjes. De pijlen wijzen naar de eenheidsmembraan, die de lichaampjes, of wat er van deze is overgebleven, begrenzen. Let op de buisvormige structuren van uiteenlopende grootte en vorm. PD = plasmodesma, W = celwand.

to those reported previously (Dijkstra and Ie, 1969; Dijkstra and Lee, 1972; Hull et al., 1969, 1970). While one part of the tissue, presumably consisting of functioning cells, contained normal bodies, the other probably defunct part was packed with anomalous bodies only.

These observations suggest that the mycoplasma abnormality described here was induced by changes in physiology of the host cells, by aging of mycoplasmas themselves, by stress caused by overpopulation in the given cell(s), or by a combination of these factors. It is also worth noting that the production of toxic materials is known in infections with certain animal mycoplasmas (Smith, 1971).

The anomalous bodies described were not artifacts caused by the procedures prior to electron microscope examination, since the same procedures were employed throughout this investigation. Such bodies were found in the sieve cells that were in process of obliteration. The latter cells may be comparable to the so-called necrotic cells. It should be pointed out that in classical literature the occurrence of necrotic cells in the sieve element was repeatedly reported as a characteristic internal symptom of 'yellows' diseased plants (Girolami, 1955; Rasa and Esau, 1961). The true nature of the necrotic cells is still unknown.

Recently, Hirumi and Maramorosch (1972) extensively studied many ultrastructural changes in presumptive degeneration of mycoplasmas of aster yellows and emphasized the importance of such a study before investigating the morphological effects of chemotherapy or other treatments of yellows agents. 'Apparently moribund mycoplasma-like bodies' found in tetracycline-treated spike-infected sandal leaf (Hull

Fig. 7. A transverse section of periwinkle stem infected with the sandal spike agent. Note the occurrence of strongly osmiophilic bodies in degenerated sieve cells. Cells in the neighbourhood are also filled with normal mycoplasma bodies. Progressive degeneration of the sieve cells and obliteration of abnormal cells are apparent. Arrows point to the abnormal deposition of callose on the secondary cell walls.

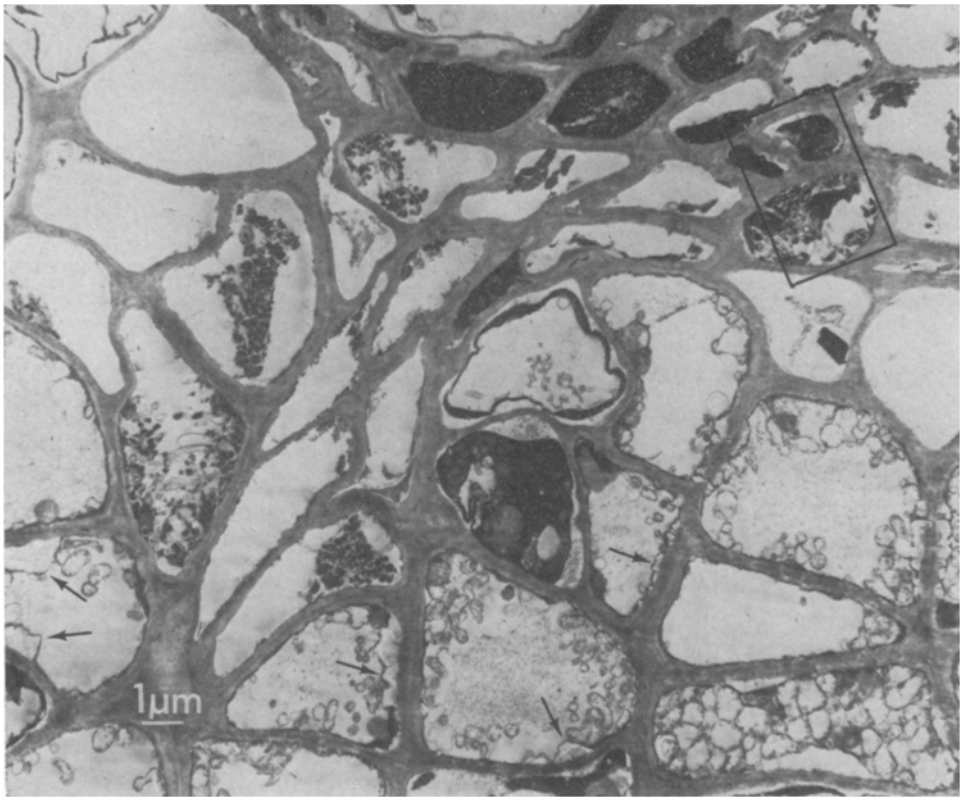

Fig. 7. Dwarsdoorsnede van een stengel van Vinca rosea, geïnfecteerd met het agens van de 'spike'-ziekte van de sandelboom. Let op de aanwezigheid van sterk osmiofiele lichaampjes in gedegenereerde zeefcellen. Cellen in de omgeving zijn ook gevuld met normale mycoplasmalichaampjes. Er treedt voortschrijdende degeneratie van de zeefcellen en te gronde gaan van abnormale cellen op. Pijlen wijzen naar de abnormale afzetting van callose op de secundaire celwanden.

et al., 1970) and some of the degenerated bodies of the aster yellows agent (Hirumi and Maramorosch, 1972) closely resemble the anomalous form described in this paper. However, it is not possible to relate significantly our finding to their observations, because neither of them gave detailed information on the location of phloem cells with the degenerated bodies and those containing normal types of mycoplasma bodies nor on the distribution of such bodies.

There is also a striking resemblance between our anomalous form (Fig. 3) and the nonviable cells of an animal mycoplasma (Maniloff, 1970) and those that were found in prolonged cultures, presumed to be an aging or death form of other animal mycoplasmas (Knudson and MacLeod, 1970).

Fig. 8. A detail of some of the phloem cells shown in Fig. 7. Note many strongly osmiophilic, anomalous bodies of different size and shapes and their membraneous materials. PD = plasmodesma, W = cell wall.

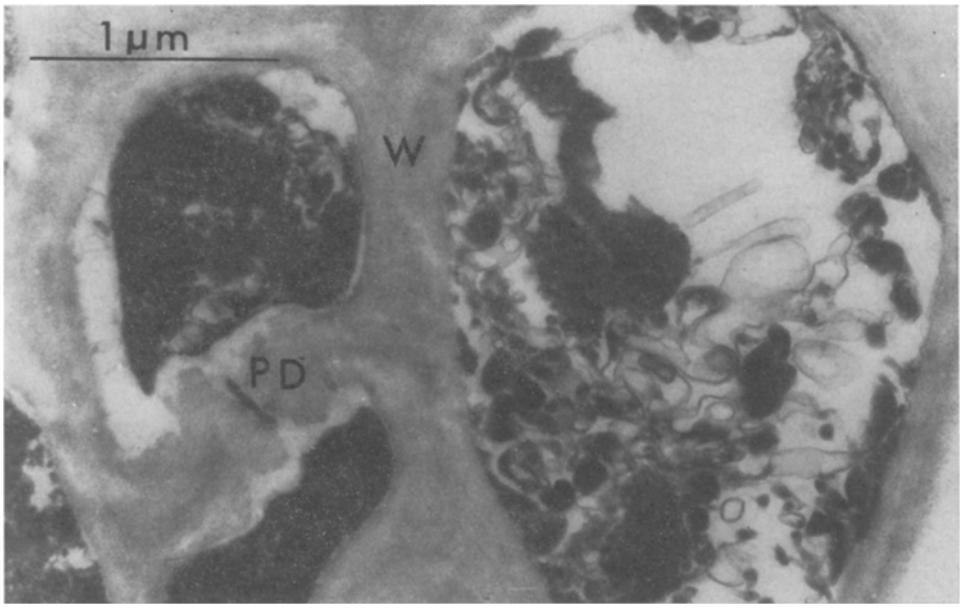

Fig. 8. Detail van enkele floëemcellen uit Fig. 7. Let op de vele, sterk osmiofiele, abnormale lichaampjes van uiteenlopende grootte en vorm en hun membraanachtig materiaal. PD = plasmodesma, W = celwand.

The cause and nature of natural degeneration of the mycoplasmas in the phloem cells are unknown and remain to be resolved by future investigations.

Samenvatting

Een abnormale vorm van de mycoplasma-achtige lichaampjes in Vinca rosea, geïnfecteerd met het agens van de 'spike'-ziekte van de sandelboom

De mycoplasma-achtige lichaampjes die in planten, aangetast door de 'spike'-ziekte van de sandelboom (*Santalum album*) worden aangetroffen, bezitten een eenvoudige structuur, bestaande uit een celmembraan, ribosomen en een DNA-streng (nucleoïd) (Fig. 1).

Ultradunne coupes van een stengel van een geïnfecteerde plant van *Vinca rosea* vertoonden drie typen pleomorfe lichaampjes, te weten: ronde lichaampjes, 160–280 nm in doorsnee, grote lichaampjes, 400–800 nm in doorsnee, en lichaampjes van 50–160 nm doorsnee met een sterk osmiofiel karakter (Fig. 3). In de lichaampjes van het eerste type ontbrak de nucleoïd en de ribosomen waren gelijkmatig verdeeld over het cytoplasma. Bij het tweede type was de nucleoïd zichtbaar in het midden van de lichaamp-

jes, maar sommige van deze hadden een elektronen-optisch leeg centrum. Van enkele van deze lichaampjes was de membraan gescheurd en er trad knopvorming en instulping van deze laatste op (Fig. 2A, 2B en 5).

De merkwaardigste structuur vertoonden de sterk osmiofiele lichaampjes, die vaak gedegenereerde cellen van zeefvaten vulden (Fig. 3–8). Deze afwijkende lichaampjes werden begrensd door een eenheidsmembraan en vertoonden geen cytoplasmatische structuur, kennelijk als gevolg van condensatie (Fig. 3 en 4). Dit type trad alleen op in bepaalde delen van aangetast floëem (Fig. 6–8). Ze wisselden sterk in grootte en vorm maar behielden een duidelijke membraan (Fig. 6 en 8).

De lichaampjes van de eerste twee typen werden, zonder de afwijkende vorm, gevonden in veel jongere floëemcellen van hetzelfde weefsel (Fig. 7).

Het is mogelijk dat de abnormale vorm, die in deze studie is waargenomen, een natuurlijk gedegenereerde vorm is van de mycoplasma-achtige lichaampjes die een rol spelen bij de 'spike'-ziekte van de sandelboom.

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Book review

Verhoeven, W. B. L.: Ziekten en beschadigingen van landbouwgewassen en hun bestrijding (Diseases and pest of agricultural crops and their control). 6th edition by H. J. de Bruin & M. Heuver. Veenman, Wageningen, 1972. 263 pp. 237 figures. Paperback. Price Dfl. 9.25.

This book by Verhoeven, a plant pathologist of the Dutch Agricultural Advisory Service, has proved its value since it first appeared in 1954.

After a short introduction on dangers of pesticides, the book describes the various diseases and pests of agricultural crops in the Netherlands with life cycles, syndromes and ways of control. The disorders are grouped alphabetically under crops. The book is profusely illustrated with representative photographs helpful for diagnosis.

Changes made since the previous edition include the ommision of some diseases and crops of decreasing importance, and the addition of some new ones. Some changes have been made in the advice on range of pesticides and the amounts to be applied.

The book is intended mainly as a practical guide for advisers and teachers in high schools and colleges, but contains valuable information for all students and other involved in plant pathology. Although the nomenclature of viruses and virus diseases is not completely up to date, the book is well prepared and presented. It certainly meets a need and is good value for money.

L. Bos