Rickettsialike Organisms: The Possible Cause of Citrus Blight in Suriname

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Citrus blight is a wilt disease of citrus known for over 90 years in Florida. The disease is characterized by zinc deficiency symptoms in the leaves, production of numerous water sprouts on the trunk and the lower branches, leaf drop and subsequent dieback. Frequently wilt starts on one limb of the tree. The disease is known under different names, such as orange blight, limb blight, dry wilt, leaf curl, roadside decline, Plant City decline, and young tree decline (Childs, 1953). Swingle and Webber (1896) and Rhoads (1936) reported that the disease was confined to Florida. Childs (1953) observed that xylem vessels of the roots and twigs of blight-affected trees were plugged with masses resembling actinomycete hyphae. These masses, observed earlier by Rhoads (1936), presumably cause the wilt by blocking of the water flow. Symptoms of a wilt disease were observed in 1975 in the Alidjan orange plantings on Rangpur lime in the Baboenhol citrus orchard in the interior of Suriname.

Light microscope studies by Power and Klas (unpublished) revealed obstructions in the xylem vessels of the roots of affected trees similar to those observed by Childs (1953). Attempts to unravel the nature of the plugs and to learn their role in blockage of water flow led to detection of rickettsialike organisms.

MATERIALS AND METHODS

Waterflow. To assess the extent that plugs retard the flow of water through xylem vessels, root pieces 2.5 cm long and 0.8 cm in diameter from healthy and blighted trees were mounted in a water-flow apparatus. This consisted of a 500-ml separatory funnel connected to a branched system of plastic tubes. A constant water level (and pressure) was maintained in the funnel from a separate 1-liter bottle connected to the separatory funnel by plastic tubing. The amounts of water collected were determined at 10, 30, 60, 90, and 180 minutes.

Light microscopy. Root pieces that showed a retarded water flow were compared microscopically with those which showed a fast flow.

Root pieces of healthy and blight-affected trees were embedded in parrafin (boiling point 58° C) and were sectioned longitudinally (15 μm thick). Paraffin was removed with xylene, and the sections examined with a Wild M-12 light microscope. Photographs were taken with a MPS 11 microcamera.

Scanning electron microscopy and x-ray diffraction. Root pieces of healthy and blight-affected trees were fixed in FAA and sent to the USDA Horticultural Research Laboratory in Orlando, Florida, for comparative scanning electron microscopic (SEM) studies. Longitudinal sections 35 microns thick were transferred to sodium cacodylate buffer (pH 7.2) and then serially dehydrated in hexylene glycol (10, 40, 60, 80, and 100 per cent). Sections were subsequently placed in a 1:1 mixture of hexylene glycol and Freon 113 for 15 minutes and transferred to pure Freon 113, three changes, 15 minutes each. Sections were critical-point dried, using Freon 113 as an intermediate fluid.

Dried sections were mounted on stubs with silver paint, sputter-coated with palladium-gold (60-40) and viewed in a JEOL JM35U microscope.

X-ray diffraction experiments were carried out to detect zinc crystals in the plugs. Plugs were picked out with needles and attached to the tip of micro glass needles. These were subsequently mounted in the camera of an x-ray diffraction apparatus consisting of a Debeye Scherer Powder Camera (diameter 115 mm), a PW 1010/30 x-ray generator operating at 40 kV and 20 mA with a copper röntgen anode, and a 1.5418A nickel filter.

The material in the camera was exposed initially to a 6-hour radiation. When no spectrum was obtained, radiation was continued for another 18 hours.

The spectral lines were calculated on the basis of the formula:

$$n.l = 2.d \sin \theta \quad (1)$$

The so-called Bragg's equation, where

l = the wavelength of radiation.
d = the spacing of a set of scattering points.
θ = the angle between the incident radiation and the scattered radiation.

A formula for the computation of d can be derived from (1):

$$d = \frac{n.l}{2. \sin \theta} \quad (2)$$

Transmission electron microscopy (TEM). Longitudinally cut root sections from a healthy-looking tree and an affected tree were prefixed in modified Karnovsky (1965) fixative (2 per cent paraformaldehyde, 3 per cent glutaraldehyde, 0.1 per cent $CaCl_2.2H_2O$ in 0.05 M cacodylate buffer, pH 7.4) for 8 days. They were postfixed in 1 per cent OsO_4 in 0.1 M cacodylate buffer, pH 7.4 for 1 hour at room temperature, rinsed in buffer and aqua bidest., and stained in 2 per cent uranyl acetate in 0.06 M Veronal acetate buffer, pH 5.1 for 1 hour at room temperature. After dehydration in increasing concentrations of ethanol, the sections were embedded in prepolymerized methacrylate + divinylbenzene, after Kushida (1961), and sectioned with a LKB Ultratome III ultramicrotome, using glass knives. The ultrathin sections were stained in 2 per cent uranyl acetate in aqua bidest., followed by staining in lead citrate, after Reynolds (1963). The specimens were viewed in a Siemens Elmiskop-101 electron microscope, operating at 80 kV.

RESULTS AND DISCUSSION

Water flow. Saturation and linear curves were obtained from the water-flow experiments. In general, the saturation curves appeared with slow water flow, whereas linearity was most frequently correlated with fast water flow.

The correlation between retarded water flow and the occurrence of obstructions in the xylem vessels of the roots was clear (table 1). Obstructions were absent or scarce in root pieces of affected trees that showed fast water flow.

The flow tended to be constant or to increase slightly (Q/min.) with time if plugs were absent in the xylem vessels, while it tended to decrease considerably if the vessels were plugged.

Roots from different sides of the tree were not plugged to the same extent, even in the heavily blighted trees. Roots on the eastern side of tree 7 in row 7 of block D-7 at Baboenhol, for example, were hardly plugged (60-360 ml/3 hr), whereas roots on the northern, western, and southern sides were heavily plugged and permitted little water flow (6-40 ml/3 hr). This observation may account for the sectorial appearance of wilt in the affected tree (Childs, 1953).

Light microscopy. The obstructions in the xylem vessels of the roots of a diseased tree are shown in fig. 1. Note that the obstructions occur on the junction of the xylem vessel elements. They were absent in a healthy-looking tree of this orchard. Apart from the gumlike or granular plugs, tyloses also play a part in the blockage of the water flow in blighted trees (fig. 2). The water-flow experiments, supported by the light-microscopic studies, prove that the wilt is caused by plugs occurring in the xylem vessels.

Scanning electron microscopy and x-ray diffraction. Scanning electron microscopy revealed the filamentous and branched structure of the plugs (fig. 3), as shown by Childs and Carlysle (1974) in roots of blighted trees in Florida. They concluded from the branching appearance that the plugs consisted of hyphae of some fungus. Serial sectioning of vascular obstructions by VanderMolen (1978) did not reveal any structure of fungal hyphae, actinomycetes, mycoplasmas or bacteria.

Transmission electron microscopy of specific filamentous plugs showed that the interior of the filaments contained a core of electron-dense material rather than cytoplasmic contents typical of fungal hyphae or bacteria (VanderMolen, 1978).

The nature of these plugs is not clear at present despite Nemec's suggestion (1974) that they were accumulations of lipid (Myelin) material. This assumption seemed unrealistic to Childs and Carlysle (1974), who presumed they were composed of polysaccharides.

The x-ray diffraction analysis showed four lines after 18 hours' radiation. The positions (d-values) of these lines are listed in table 2. The lines correspond to the standard spectral lines of crystalline silica, which suggests that silica occurred in the plugs. This does not indicate that the plugs consist of silica crystals. Since spectral lines were not observed during the first 6 hours, it is obvious that the obstructions are of an amorphous nature. The silica spectral lines may, therefore, have been caused by an erratic deposit of silica, since the Baboenhol soils are highly siliceous.

Zinc crystals were not detected during the x-ray diffraction analyses here or by Childs in Florida (personal communication). However, Smith (1974) and Wutscher *et al.* (1977) detected accumulation of zinc in the wood of blight-affected trees.

Transmission electron microscopy. Transmission electron microscopy revealed pronounced outgrowths of the edges of perforation plates of diseased trees (fig. 4). Similar structures were also observed by VanderMolen (1978). These outgrowths were not observed in healthy trees.

Masses of bacterialike organisms with rippled cell walls were recognized upon further examinations of diseased tissues (fig. 5). They ranged from 0.2 to 0.77 μm in diameter and 1.14 to 2.1 μm in length, a size range comparable to that reported by Feldman *et al.* (1977). Electron-dense areas found in the organism may be concentrated areas of nucleic acid. DNA-microfibrils in the nucleoid area of the prokaryote bodies are clearly seen in the electron-lucent regions of figs. 5A and 5B. It is clear that the cell wall of the organism consists of at least three layers (fig. 5B). The inner plasmalemma membrane is closely surrounded by an outer cell wall of rippled texture. The thin, translucent middle layer separating the plasmalemma from the cell wall, however, could be a fixation artifact.

The membranous structure of the cell wall suggests "gram-negative" properties and, probably, also a sensitivity to tetracyclines. The rippled nature of the cell walls, which is one of the main characteristics of rickettsialike organisms, can clearly be observed near the ends of the organism (fig. 5B).

These properties suggest that we are dealing with a rickettsialike organism. However, in contrast to the observations of Feldman *et al.* (1977), the organism originating from Baboenhol is strongly vacuolated. It appears that the rickettsialike particles are present in the trees long before symptom expression starts, since they have been observed in a healthy-looking tree which became diseased later.

Similar results were also reported by Feldman *et al.* (1977). It is likely that the rickettsialike organisms induce the production of gumlike or granular obstructions near the junctions of xylem vessel elements, since Pierce's disease, which was shown to be caused by similar associated organisms (Davis *et al.*, 1978), is accompanied by brownish gum and tyloses in the xylem vessels (Houston *et al.*, 1947).

To study the pathogenicity and the transmissibility of the disease, approach graft experiments have been started.

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TABLE 1
WATER-FLOW DATA OF A HEALTHY AND A BLIGHT-AFFECTED TREE FROM THE BABOENHOL CITRUS ORCHARD

Time (in min)	Healthy				Blighted			
	R-1*	R-2	R-3	R-4	R-1	R-2	R-3	R-4
	Q†	Q	Q	Q	Q	Q	Q	Q
10	26	44	14	16	3	4	7	17
60	152	224	76	142	9	8	14	62
180	382	580	234	552	14	12	26	104
Plugs Present	—	—	—	—	+	+	+	+

* R-1 = root piece No. 1, etc.
† Q = quantity of water collected (in milliliters).

TABLE 2
POSITIONS OF SPECTRAL LINES, OBTAINED FROM X-RAY DIFFRACTION OF PLUGS OCCURRING IN XYLEM VESSELS OF BLIGHT-AFFECTED TREES

Line No.	$d = \frac{l}{2 \sin \theta}$	Intensity I	Standard d-values for Silica
1	4.26	60	4.25
2	3.34	100	3.34
3	2.45	30	2.45
4	1.82	50	1.82

Fig. 1. Plugs occurring on the junctions of the xylem vessel elements of roots from tree no. 5 in row 7 of block D-2 of the Baboenhol citrus orchard.

Fig. 2. Occurrence of tyloses in a blight-affected tree.

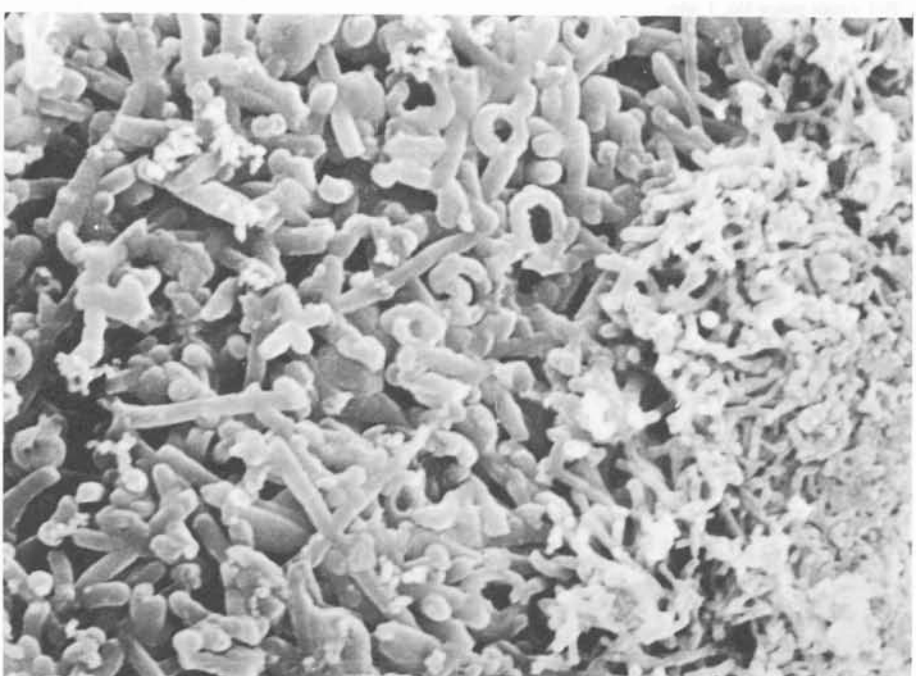

Fig. 3. Scanning electron micrograph of the plug material. Note the filamentous and branching appearance.

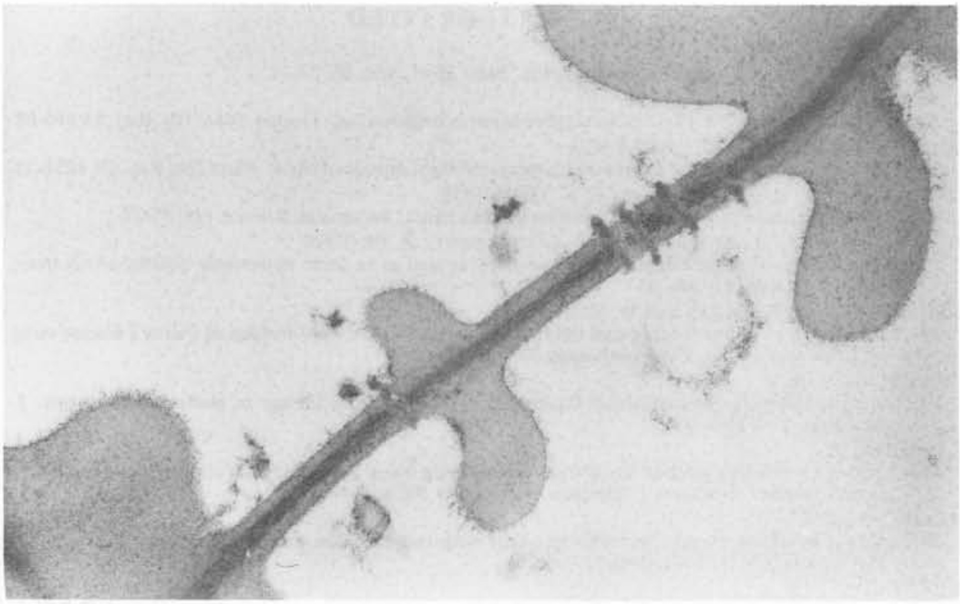

Fig. 4. Outgrowths on the wall of perforation rim.

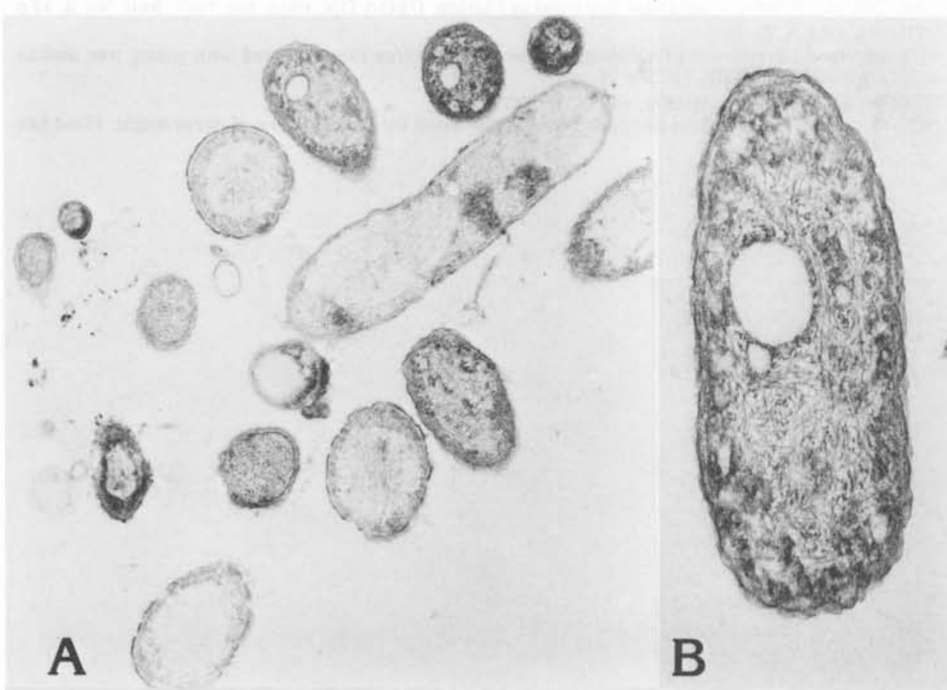

Fig. 5. A) Rickettsialike organisms associated with blight. Note the rippled cell walls.

Fig. 5. B) Enlarged rickettsialike organism. Note the rippled cell walls near the ends and at least three layers in the cell wall.

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