Phytomonas staheli Associated with Coconut and Oil Palm Diseases in Colombia

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ABSTRACT

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Phytomonas staheli was observed in tissues and in sap expressed from diseased coconut and African oil palms from three widely separated regions of Colombia. Light and transmission electron microscopy indicated these organisms in sieve tube elements of the phloem of root, bud, and inflorescence tissues. Flagellates were present in dwarf coconut palms with lethal wilt in Tumaco on the Pacific coast by the border with Ecuador, in mature African oil palms affected by sudden wilt disease (marchitez sorpresiva) in the Amazon basin, and in immature African oil palms with the case nine (caso nueve) syndrome in north central Colombia. These organisms were morphologically indistinguishable from *P. staheli*, thus extending the geographic range of this suspected plant pathogen. Preliminary attempts to culture these organisms failed.

The occurrence of flagellated protozoa in plants has been widely documented for the laticifer-inhabiting organisms *Phytomonas davidi* Lafont and *P. elmassiani* Migone (4,9,10). Although some investigators have suggested that these organisms may be pathogenic to their plant hosts, no definitive studies have proved pathogenicity, and infected plants generally show no deleterious effects (3,9). However, in 1931, Stahel, studying the phloem necrosis disease of coffee (*Coffea liberica*) in Surinam, found flagellates in hyperplastic phloem of affected plants (15). He reported that the disease is graft transmissible and that the associated flagellate, *P. leptovasorum* Stahel, is invariably associated with symptomatic plants. This flagellate was not amenable to culture and, although Koch's postulates were not fulfilled, it is the most likely pathogen.

Stahel's work was largely overlooked by plant pathologists, even though confirmed by Vermuelen in 1963 (19). In 1976, Parthasarathy et al (13) reported flagellates of the genus *Phytomonas* in coconut palms (*Cocos nucifera*) affected by hartrot disease in Surinam. These flagellates, like *P. leptovasorum*, were present in living sieve elements of the phloem. McGhee and McGhee (11) collected samples of the coconut palm organism in Surinam and from morphological determinants named it *P. staheli*. Although primary cultures of this organism were obtained in a few instances, attempts to continuously culture *P. staheli* in diphasic blood agar media failed. Although Koch's postulates for pathogenicity have not been fulfilled, *P. staheli* remains the most likely causative agent of hartrot due, in part, to its obligate intracellular habitat in infected palms.

The reports of flagellates in Surinam resulted in the initiation of several investigations on the nature of several similar palm diseases. Dollet et al (1) reported the occurrence of flagellates in sudden wilt disease (marchitez sorpresiva) of African oil palm in Peru, and Thomas et al (16) reported that flagellates are associated with this palm disease in Ecuador. Later reports confirmed the presence of flagellates in dying coconut palms in Ecuador and Colombia and in African oil palm in Colombia (2,6,8) and Surinam (18).

This paper describes the morphology of phytomonads obtained from three distinct palm diseases in widely separated regions of Colombia and ascribes these to *P. staheli*.

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MATERIALS AND METHODS

Collection of phytomonads. Palm tissues from three sites throughout Colombia were brought to the Plant Virology Laboratory of the Instituto Colombiano Agropecuario within 2 days of collection. Root, bud, and inflorescence tissues were examined for flagellates by expressing sap onto a microscope slide and observation under phase contrast illumination at ×400. Slides on which living phytomonads were observed were air dried, fixed in methanol, and stained with a 1:40 dilution of Giemsa at pH 7.2 as described elsewhere (11). Slides were counterstained 3 days at room temperature in 0.1% safranin-0 to enhance visibility of nuclei. Length, width, and distances from kinetoplast and nucleus to the anterior tip of the cells were measured for 50 flagellates each from diseased plants at ×1,000.

Light and electron microscopy. Tissues confirmed to contain flagellates by phase contrast microscopy were fixed at pH 7.4 in 0.1 M collidine-buffered 2% paraformaldehyde and 2% glutaraldehyde for 24 hr and placed in 0.1 M collidine buffer for transport to Florida. Postfixation procedures, staining, and embedding in Spurr's plastic were performed as described elsewhere (16). Sections 1 μm thick were cut on an LKB ultramicrotome for light microscopy and stained in 1% aqueous cresyl violet acetate 2–5 min. Ultrathin sections were cut for transmission electron microscopy and examined in a Phillips EM 201 microscope.

Culture. Sap containing living flagellates was expressed from tissues of each of the three diseases examined and placed in SP-4 (7,17) culture broth. Broth samples were examined at intervals with the phase contrast microscope to determine if the flagellates had multiplied.

RESULTS

Symptoms of palm diseases. All three disease syndromes were characterized by rapid browning and desiccation of foliage, rot of roots and bud tissues, and abortion of fruit in mature palms. The palms generally died within 2 mo after the onset of symptoms.

Wilt-affected dwarf coconut palms near Tumaco on the Pacific Coast just north of Ecuador contained flagellates (6). Leaf necrosis began with the oldest fronds at the base of the crown and extended upward into the crown, affecting successively younger fronds. Foliar necrosis was associated with nutfall and inflorescence necrosis, similar to that described for hartrot of coconut palm in Surinam (13). Field observations indicated that this problem affected only dwarf cultivars (locally called "Manila"); no tall cultivars ("Panama Tall") were affected. Also no oil palms in the region were observed to have symptoms.

African oil palms with sudden wilt near Villavicencio in the Llanos region (the Amazon basin) also contained flagellates. The symptoms were as described for this disease in Ecuador (16). Only mature, bearing palms, 5 or more years old, were affected. Foliar discoloration begins in the lowest fronds, all fruit aborts and rots, and leaves desiccate and die from oldest to youngest, moving upward in the crown. Discoloration begins at the leaf tips and extends toward the leaf bases.

Table 1. Dimensions (μm)[a] of *Phytomonas* cells from various sources in Colombia and of authentic *P. staheli* cells

Source	Length	Width	Anterior end to	
			Kinetoplast	Nucleus
Coconut[b]	17.3 ± 1.5	1.0	2.8 ± 0.5	6.0 ± 1.0
Petiole base	16.5 ± 1.2	1.0	2.7 ± 0.5	5.9 ± 0.8
Roots	17.8 ± 1.5	1.0	2.9 ± 0.4	6.2 ± 0.6
Oil palm[c] (petiole base)	20.1 ± 1.5	1.0	2.0 ± 0.4	5.5 ± 0.5
Oil palm[d] (root)	21.7 ± 2.6	1.0	2.7 ± 0.5	6.4 ± 0.6
P. staheli (11)	20.4 ± 3.2	0.6	1.7 ± 0.4	6.2 ± 1.2

[a] Means and standard deviations of 50 cells from each source measured at ×1,000.
[b] Dwarf coconut palms dying in southwest Colombia along the Pacific Coast (Tumaco).
[c] Sudden wilt diseased African oil palms from the Amazon basin of Colombia.
[d] Case nine disease of African oil palm near Barranca Bermehas in central Colombia along the Rio Magdalena.

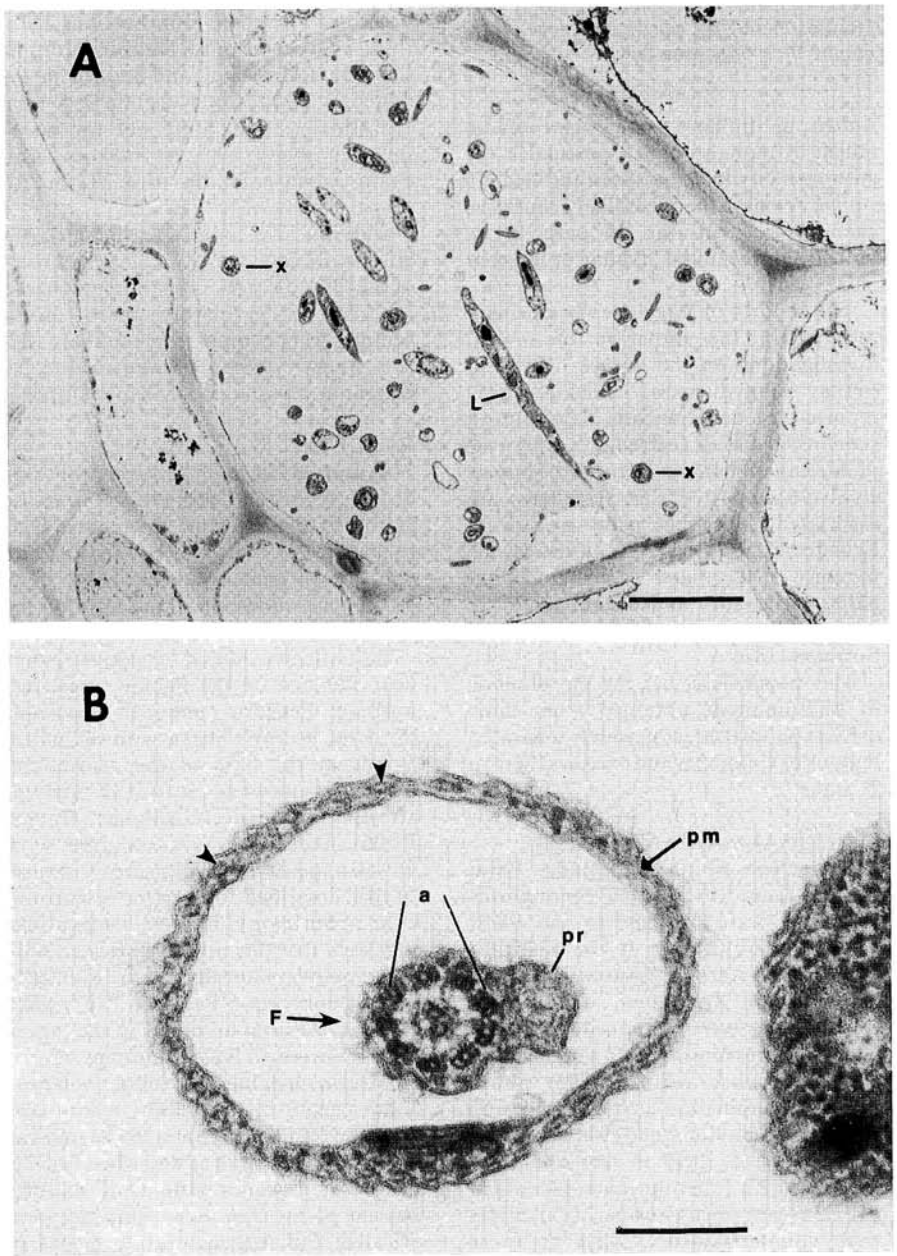

Fig. 1. Electron micrographs of *Phytomonas staheli* in sieve tube elements of wilt-diseased coconut palms from Tumaco, Colombia: **(A)** Low magnification showing both cross (x) and longitudinal (L) sections of flagellates (bar = 5 μm). **(B)** Cross section through flagellar pocket at anterior end of a *Phytomonas* cell. Note microtubules (darts) in pellicular membrane (pm). The single flagellum (F) consists of an axoneme (a) of parallel microtubules and a paraxial rod (pr) (bar = 0.2 μm).

Buds and roots of African oil palms with case nine disease were collected near Puerto Wilches in north central Colombia in the Rio Magdalena valley. Case nine disease affects only nonbearing, immature palms younger than 3 yr; mature palms in the same region are not affected. Foliar discoloration begins in the center of individual leaves rather than at leaf tips. Spear leaf necrosis develops before all leaves have turned brown, and most roots become necrotic as the foliage dies. No inflorescence or fruit necrosis was noted as none of these palms were of bearing age.

Dimensions of flagellates. Dimensions of photomonads from each of the three palm diseases and *P. staheli* are given in Table 1. No significant differences were discernible among the three photomonads found in palms in Colombia or from *P. staheli* as described by McGhee and McGhee (11).

Light microscopy. Photomonads were observed only within sieve tubes of the phloem. Not all sieve elements contained flagellates, and the numbers of flagellates varied among infected cells. No flagellates were seen in any other cell type. All flagellates were typical elongated promastigotes.

Electron microscopy. Ultrastructure of the phloem flagellates was typical for the genus *Phytomonas* (Fig. 1). Cells were bound by a continuous membrane extending into the flagellar pocket for the single anterior flagellum. A single layer of microtubules extended beneath the pellicle and spiraled along the longitudinal axes of the cells. Nuclei were located anteriorly behind the kinetoplast, which was flattened with its long axis perpendicular to that of the cell. No significant ultrastructural differences were noted among flagellates from the three regions.

Culture. No multiplication of palm flagellates was detected. Some tubes contained motile photomonads 14 days after inoculation of broth, but no multiplication was demonstrated and transfers to fresh broth then did not result in growth.

DISCUSSION

Because no significant morphological differences were noted among the three sets of flagellates sampled from Colombia and *P. staheli* (11), we conclude that all belong to the same species.

Identification of *P. staheli* in coconut and oil palms in Colombia extends the known range of this organism from Surinam to the Pacific Coast and the Amazon basin. The sudden wilt of oil palms in Ecuador (16) and Peru (1) and the Cedros wilt of coconut palm in Trinidad (20), probably coidentical to hartrot, indicate further extension of the range of this flagellate, although no cellular measurements have been reported from these countries.

The wilt disease of coconut palms in

Tumaco is very similar to descriptions of hartrot in Surinam (13,14). The differences between sudden wilt and case nine diseases in African oil palm are difficult to explain, however, in light of the similarity of the presumptive pathogens. Certainly, different races of *P. staheli* could exist that might produce a different series of symptoms in infected palms. The fact that one disease affects only mature palms and the second only young palms could indicate a vector feeding preference, with different vectors existing in the two locations. Differences in site and host cultivar also could affect symptom development.

Although an insect is presumed to carry these phloem-inhabiting phytomonads from plant to plant, no vector has yet been demonstrated. However, the phloem habitat of these presumptive pathogens suggests that phloem-feeding homoptera would be the most suspect insect group. *P. davidi* and *P. elmassiani* both have hemipteran vectors, and these insects introduce the flagellates into the latex vessels of their plant hosts (9,10). The most likely vectors for the phloem-inhabiting flagellates are the insects that feed specifically in the phloem. The leafhoppers particularly should be investigated as they are known carriers of other phloem-restricted plant pathogens (5).

Although limited preliminary attempts to culture *P. staheli* have failed, the successful isolation of a strain of *P. davidi* by McGhee and Postell (12) indicates that this should not be an impossible task. Future research should concentrate on the cultural and serologic identification of these probable plant pathogens, on identification of their vectors, and on proving pathogenicity through Koch's postulates.

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