Electron Microscopy of Flagellated Protozoa Associated with Marchitez Sorpresiva Disease of African Oil Palm in Ecuador

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ABSTRACT

THOMAS, D. L., R. E. McCOY, R. C. NORRIS, and A. S. ESPINOZA. 1979. Electron microscopy of flagellated protozoa associated with marchitez sorpresiva disease of African oil palm in Ecuador. Phytopathology 69:222-226.

Mature sieve elements of oil palms from Ecuador with marchitez sorpresiva (sudden wilt) disease contained uniflagellated protozoa that were classified in the genus *Phytomonas* of the family Trypanosomatidae. Phloem necrosis was not evident and distribution of the flagellates was uneven in diseased palms. Although the etiologic role of the protozoa was not determined, our study supports previous research hypothesizing that trypanosomatid protozoa are involved in the marchitez sorpresiva disease syndrome.

Additional key words: Elaeis guineensis L., phloem, *Phytomonas*, sudden wilt.

Marchitez sorpresiva (sudden wilt) was first reported in 1963 as a disease of African oil palm (*Elaeis guineensis* L.) on a Colombian plantation where over 65% of the trees were lost to the disease in the following 12 yr (13). Within 10 yr of this first major outbreak, the disease had spread throughout much of Colombia and into Peru and Ecuador (13,27). Early symptoms of marchitez sorpresiva include loss of fruit luster followed by rotting of fruit and cessation of flowering (Fig. 1). Concurrently, foliage discoloration and desiccation begin at the tips and progress to the bases of the lower leaves (Fig. 2). The brown discoloration advances upward into the crown over a period of approximately 2 mo. Spear leaves frequently fail to open and may turn necrotic and collapse, with a secondary soft rot descending toward the meristem. Root rot is concurrent with inflorescence and foliar symptoms, and palms usually die within 3 mo of the first visible symptoms.

Research has failed to show that viruses, bacteria, fungi, mycoplasmas, or parasitic nematodes are etiologic agents of marchitez sorpresiva (13,14,27). However, because insecticides controlled the disease somewhat, it was postulated that insects either were vectors of the disease (27) or were able to predispose the palms to invasion by some biotic agent (13). In 1977 Dollet et al (3) observed flagellated protozoa of the family Trypanosomatidae in the sieve tubes of diseased oil palms from Peru, and they postulated that the flagellates had a role in the marchitez sorpresiva disease syndrome.

Infection of plants by trypanosomatid protozoa was first reported in 1909 with the description of *Phytomonas* (*Leptomonas*) *davidi* from the latex of an *Euphorbia* species (11). Early European literature reported widespread presence of latex-inhabiting trypanosomatids in many *Euphorbia* species (2), but most of the research on latex protozoa has concentrated on the nonpathogenic (9,30) *P. elmassiani* that commonly invades plants of the family Asclepiadaceae (6,17,30) and is transmitted by lygaeid insects (8,10,15).

The first trypanosomatid reported to be associated with a plant disease was *P. leptovasorum*, a graft-transmittable, sieve element inhabitant of coffee (*Coffea liberica*) with phloem necrosis in Surinam (24,25,26,29). Phloem-inhabiting *Phytomonas* protozoa have since been found in association with hartrot disease of coconut palm (*Cocos nucifera*), oil palm, and maripa palm (*Maximiliana maripa*) (19,20,22). The report of marchitez sorpresiva in Peru by Dollet et al (3) is another record of phloem-inhabiting trypanosomatids associated with a disease. Although they did not determine the pathogenicity of the flagellates, they felt that such a hypothesis would be strengthened if similar protozoa were observed in diseased oil palms from other geographic regions. The presence of flagellates in oil palms from Ecuador with marchitez sorpresiva disease was confirmed in a preliminary report by Thomas et al (28). This investigation presents further electron microscopic details.

MATERIALS AND METHODS

Tissues from three oil palms, two with and one without marchitez sorpresiva symptoms, were collected from areas near Santo Domingo de los Colorados, Ecuador. Samples were excised from unopened inflorescences,unemerged leaf bases, and areas of the trunk within 15 cm of the apical meristem. The specimens were fixed at room temperature in pH 7.4, 0.1 M collidine-buffered 2% paraformaldehyde, 2% glutaraldehyde for 48 hr and then stored in 0.1 M collidine buffer at ambient temperature for transport to Florida. Twelve days after sampling, the specimens were postfixed in collidine-buffered 2% osmium tetroxide for 6 hr, transferred to 0.5% aqueous uranyl acetate for 18 hr, and dehydrated in a graded ethanol-acetone series, all at 4 C. The specimens were embedded in Spurr plastic (23), thin-sectioned with glass knives on a LKB Ultratome III, collected on Formvar-coated grids, poststained with uranyl acetate and lead citrate, and then examined with a Philips EM 201 electron microscope at either 60 or 80 KV. Specimens from six additional control oil palms were collected from a plantation in Panama where marchitez sorpresiva had not been reported. The collections, specimen handling, and examination were similar to those of the Ecuadorian material except the initial aldehyde fixation was for 18 hr and there was no prolonged specimen storage in buffer. A total of 197 vascular bundles from diseased trees and 200 vascular bundles from control trees were examined. For size measurements of protozoa with a light microscope, 4-μm sections of the embedded material were poststained with 1% aqueous crystal violet and viewed at $\times$ = 1,000, 1,600, or 2,000.

RESULTS

Uniflagellated protozoa were observed in the phloem of both oil palms with marchitez sorpresiva symptoms but not in that from the seven palms without the disease. Although all samples were

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collected from relatively young parts of the palms, tissues of different ages were examined. No correlation between tissue age and protozoa concentration was found. Flagellates were observed only in mature sieve elements of the protophloem and metaphloem, and their distribution was uneven. Of over 100 randomly chosen vascular bundles from diseased palms, only 17% contained protozoa, and about 50% of the sieve elements within an invaded vascular bundle contained the organisms. An average of seven, with a range of 3–15, flagellates were counted from cross sections of invaded sieve elements, but occasionally protozoan bodies completely filled the sieve elements (Fig. 3).

When sectioned in a favorable plane, the protozoa showed the structure of typical trypanosomatid promastigotes (7) with elongated bodies (Fig. 4). A maximum length for the protozoa was not determined but a range of 5.2–10.5 μm, excluding flagella, was observed when thick sections were viewed with a light microscope. Diameters of 0.5–1.6 μm were observed from the same thick sections.

Individuals were bounded by a continuous membrane that extended into the flagellar pocket and surrounded the single anterior flagellum, forming the flagellar sheath (Fig. 5). Each protozoan contained a single layer of parallel and approximately equidistant microtubules that spiraled beneath the pellicle along the longitudinal axis (Fig. 6). Most cells had scattered, membrane-bounded dense bodies (Figs. 4 and 6) similar to glycerophosphate oxidase bodies (1). Nuclear interiors stained uniformly and were of moderate density except for denser nucleoli and occasional aggregations of chromatin (Fig. 6). The nucleus was located in the anterior part of each flagellate behind a single kinetoplast that was limited by a double membrane. The kinetoplast appeared as a flattened, disk-shaped organelle with its longitudinal axis perpendicular to that of the organism (Fig. 5). A nucleoid, consisting of an electron-dense, distinct band of anteroposteriorly oriented DNA fibers, was located at the center of the kinetoplast. The structure of the flagellum, including the axoneme and paraxial rod (Fig. 7), was similar to that described for *P. elmassiani* by Paulin and McGhee (21). Flagellates undergoing binary cellular division were observed occasionally (Fig. 8).

In addition to elongated protozoa, globose forms, which probably were artifacts caused by the long storage in buffer, were observed throughout much of the examined material. Another probable fixation artifact was a second type of kinetoplast that was more ovoid and had a more diffuse nucleoid than the flattened kinetoplasts.

Infrequently, protozoa appeared to be moving through sieve plates, but whether this resulted from the organism's motile ability or whether the flagellates were pulled into the pores with the sudden release of phloem pressure when the specimens were cut from the palms before fixation was not determined. Fine filaments of P-protein (4) were sparsely distributed throughout sieve elements containing protozoa, and although these cells contained a peripheral zone of cellular contents common to differentiated palm sieve elements (18), the amount of these components was less than in sieve elements from the same sections that did not contain protozoa. No phloem necrosis was noted in any of the specimens.

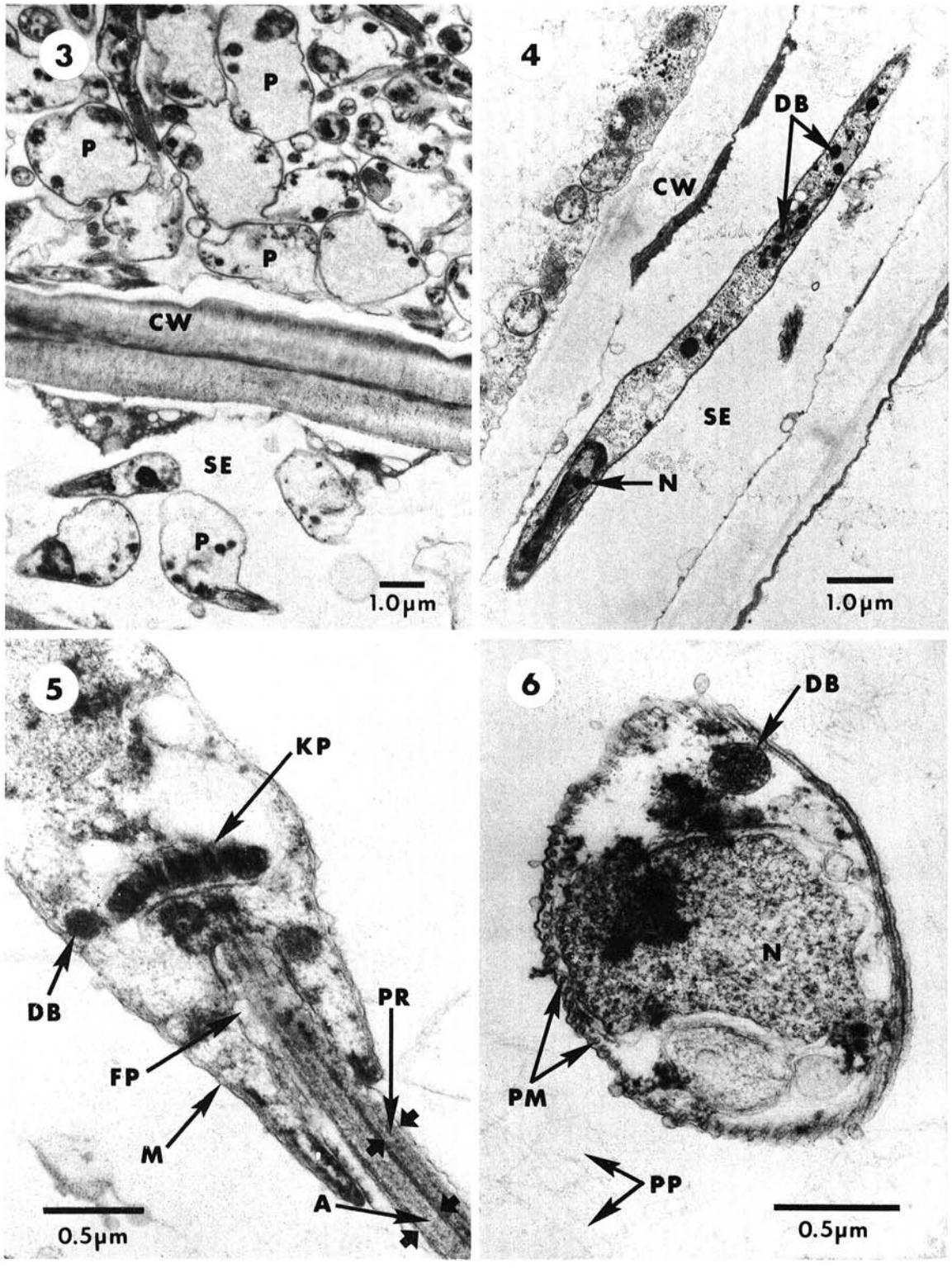
DISCUSSION

On the basis of morphology and structure, the flagellates were classified in the family Trypanosomatidae (12). Because *Phytomonas* is the only genus that includes plant inhabitants

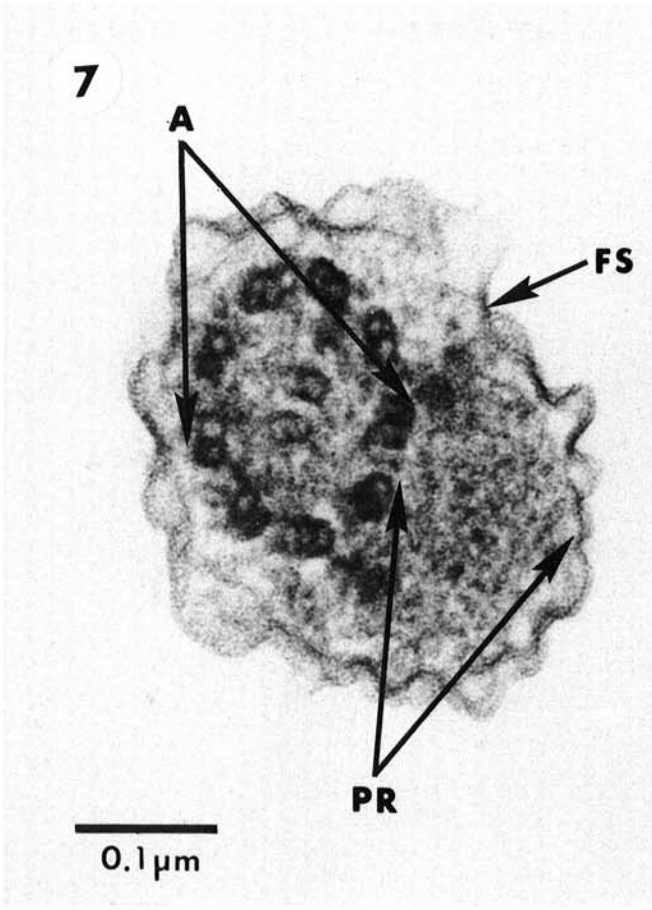
Figs. 1 and 2. 1) Fruit cluster surrounded by petiole bases (PB) from an African oil palm infected with marchitez sorpresiva. The individual fruits (arrows) have rotted. **2)** African oil palm with moderately advanced symptoms of marchitez sorpresiva. Older leaves (below arrows) have turned brown.

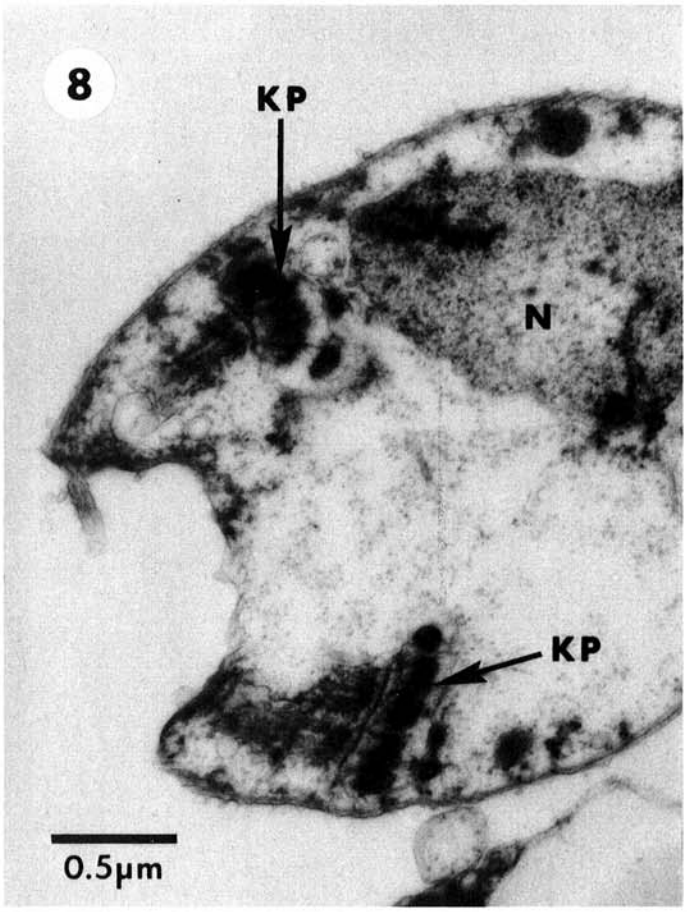
within this family (12), the protozoan observed in our study was tentatively placed in this genus. In light of research questioning the validity of using protozoan size or host for determining species of *Phytomonas* (5,6,16), no attempt was made to determine the species of this protozoan.

It is not possible to conclude from this study that flagellated protozoa are the etiologic agents of marchitez sorpresiva because the number of palms sampled was limited and no culture or transmission experiments were attempted. However, our work supports the results of Dollet et al (3), and since flagellates were associated



Figs. 3–6. 3) Longitudinal section through two sieve elements (SE) of oil palms with marchitez sorpresiva disease showing apparent differences in the concentration of protozoa. P = protozoan, CW = cell wall. **4)** Longitudinal section through a sieve element (SE) and a portion of an elongated protozoan. N = nucleus, DB = dense body, CW = cell wall. **5)** Longitudinal section through the anterior portion of a protozoan with a disk-shaped kinetoplast (KP) whose nucleoid consisted of an electron-dense, distinct band of DNA fibers. DB = dense body, FP = flagellar pocket, PR = paraxial rod (between darts) of flagellum, A = axoneme (between darts) of flagellum, M = pellicular membrane. **6)** Oblique section through a protozoan showing pellicular microtubules (PM) beneath the pellicular membrane. The tubules are seen in transverse section at the left of the photomicrograph, and their spiraling can be seen around the remainder of the cell. N = nucleus, DB = dense body, PP = P-protein.





Figs. 7 and 8. 7) Transverse section through the distal portion of a flagellum composed of an axoneme (A) and a paraxial rod (PR) surrounded by a flagellar sheath (FS). **8)** Longitudinal section through a dividing protozoan. KP = kinetoplasts, N = nucleus.

with the disease from another geographic area, evidence has been added to the hypothesis that protozoa are involved in the marchitez sorpresiva disease syndrome. Although isolation and transmission studies would be necessary to confirm the etiologic role of these trypanosomatids, further evidence for their involvement might be gained by treating diseased palms with chemotherapeutants, such as aromatic amidines, quinoline pyrimidines, and phenanthridinium compounds, that have specific activity against related trypanosomatids.

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