investigators also observed aflatoxin production by an isolate of strain 1988 obtained from other sources but were unable to repeat the finding. The culture possessed the gross colony appearance, the morphology and size of the conidial structures, and the cultural response on Czapek's medium characteristic of *A. oryzae*. However, 11-day-old cultures of this microorganism exhibited a deep green color and spore dimensions and patterns that are not characteristic of *A. oryzae*.

In summary, it has been shown that aflatoxin accumulation results from the growth of a variant strain of *A. oryzae* (NRRL strain 1988) on cowpeas. Aflatoxin was also produced by this organism growing on rice, but not on soybean. These findings lead us to conclude that this strain is a variable one. While NRRL 1988 does not produce aflatoxin during fermentation of soy sauce, it appears to have the capability of toxin production on other substrates. The use of this strain of *Aspergillus oryzae* for food fermentation and production of enzymes as food processing aids should be reexamined in light of these findings.

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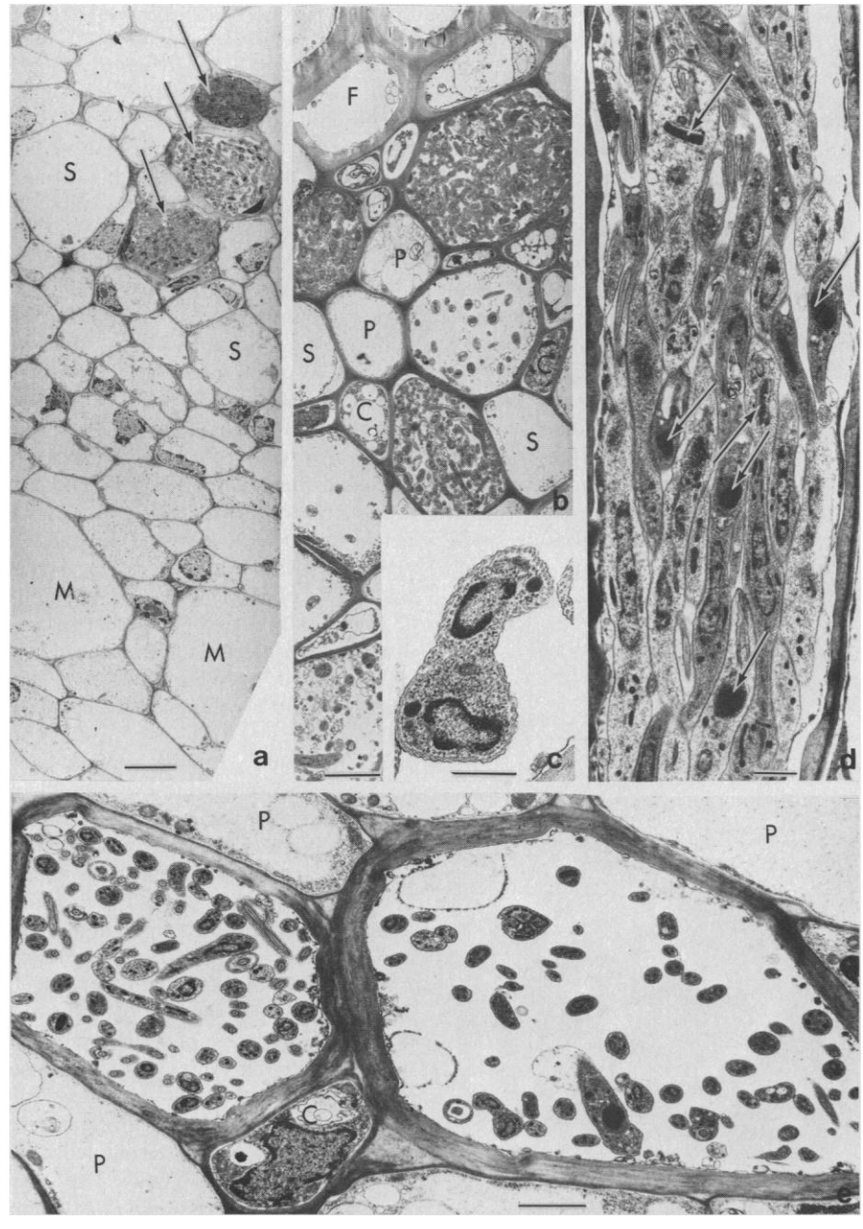
12. We thank N. F. Haard, Rutgers, for his invaluable cooperation and support; S. Hagan, Agricultural Research Service, U.S. Department of Agriculture, for her assistance with aflatoxin assays; W. Hesseltine of Northern Utilization Research and Development Division, U.S. Department of Agriculture, Peoria, Ill., for his cooperation, interest, and for supplying cultures of NRRL strain 1988; J. Peterson, Rutgers, for his identification of the variant cultures of *Aspergillus oryzae*; and to J. Ayres and T. Hamsa, University of Georgia, for their identification of the microorganism. This is a paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers—The State University of New Jersey, New Brunswick.

21 January 1976; revised 8 March 1976

Trypanosomatid Flagellate in the Phloem of Diseased Coconut Palms

Abstract. *Ultrastructural observations of the phloem of coconut palms affected by "hartrot" disease in Suriname have revealed the presence of the plant-infecting flagellate* Phytomonas *in mature sieve tubes. The occurrence of these flagellates during the earliest symptoms of the disease and the correlated increase and spread of the flagellates in the phloem as the disease progresses suggest that the organisms may be pathogenic to the palms.*

Although the existence of the plant-infecting trypanosomatid flagellate *Phytomonas* (*1*) has been known for nearly 70 years, relatively little is known about the flagellate's relationship to its hosts. The flagellate has been found chiefly in laticiferous plants (*2*), and in those plants it is apparently confined to the latex-bearing cells—the laticifers (*3*). Most investigators suggest that the flagellate is nonpathogenic to its laticiferous plant hosts (*2*, *4*). However, a few reports from Europe and elsewhere do suggest that the flagellate may be pathogenic to some of its latex-bearing hosts (*5*). The lygaeid hemipteran *Oncopeltus* is known to be



the insect host that commonly transmits the flagellates to milkweeds (*6*).

The first report of *Phytomonas* infection in a nonlaticiferous plant was made more than 45 years ago by Stahel (*7*), who demonstrated its presence in the sieve tubes (*8*) of coffee plants (*Coffea liberica*) suffering from a wilt disease in Suriname. We now report a second incident of a trypanosomatid infection in a nonlaticiferous plant, and a ultrastructural study, perhaps the first, of the plant-infecting flagellates in situ.

Our findings also suggest that the trypanosomatid is associated with "hartrot" (*9*), a disease that has killed thousands of economically important coconut palms in Suriname.

Samples of young leaves and inflorescences from eight coconut palms (*Cocos nucifera*) that showed different stages of hartrot were collected at three different localities in Suriname together with comparable samples from three healthy ones. Our electron-microscopic observations of phloem have revealed the presence of flagellated organisms in mature sieve elements of the diseased palms but none in the healthy ones. The flagellates were present only in the enucleate mature sieve elements and not in any nucleate cell of the host (Fig. 1, a, b, and e). Samples from palms in advanced stages of the disease had flagellates in 10 to 100 percent of the mature sieve elements in all the vascular bundles examined (*10*). Many sieve tubes of the protophloem and early metaphloem were plugged by the flagellates (Fig. 1, a, b, and d). The flagellates were nucleate, 1.0 to 2.5 μm in diameter and 12 to 18 μm in length, were uniflagellate, and were usually oriented longitudinally within sieve tubes (Fig. 1d). On the basis of size, morphology, and ultrastructure, the flagellate could be readily classified as a mastigophoran protozoon belonging to the order Kinetoplastida, family Trypanosomatidae, genus *Phytomonas*. The flagellates in the palm sieve tubes are similar in size range and in morphology to *P. davidi* Lafont (*11*) and *P. elmassiani* Migone (*12*, *13*) that infect laticiferous plants and to *P. leptovasorum* Stahel found in the phloem of diseased coffee plants (*7*). Since the criteria for establishing species for this genus are still a problem (*13*), further studies are needed before we can determine the species of *Phytomonas* that infect the coconut palms.

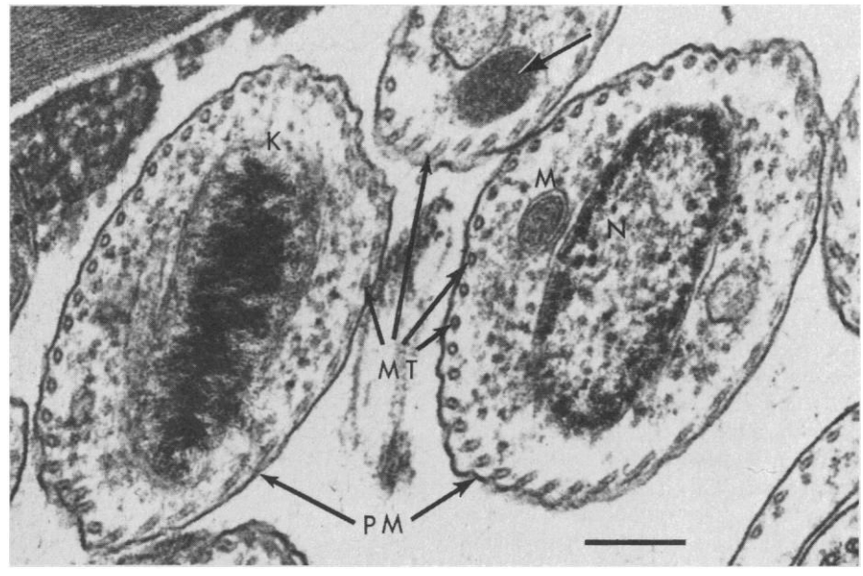


Fig. 2. Transverse section of the flagellates showing the pellicular membrane (*PM*), pellicular microtubules (*MT*), mitochondrion (*M*), nucleus (*N*), kinetoplast (*K*), and an opaque granule (unlabeled arrow) (scale bar, 2000 Å).

The ultrastructure of the flagellates in general was similar to that of *P. elmassiani* which infects milkweeds (*14*). A row of pellicular microtubules is present, adjacent to the pellicular membrane (Fig. 2). The microtubules are oriented parallel to the longitudinal axis of the organism. Transverse section of the flagellar axenome at the distal portion exhibits the typical nine groups of two microtubules. The structure of the nucleus and the size of the DNA portion of the kinetoplast (*15*) of *Phytomonas* (Figs. 1d and 2) in palm sieve tubes were not identical with those of *P. elmassiani*. Chromatin in the nuclei of the flagellates we examined was usually concentrated at the periphery of the nucleus. Well-defined nucleoli were observed in only a few of the organisms. The DNA band of the kinetoplast was about twice the width of that seen in *P. elmassiani* (*14*). As reported in *P. elmassiani*, no Golgi bodies were evident in the organisms. Several electron-opaque bodies of unknown nature were usually present in the cytoplasm (Fig. 2). Transectional views of flagellates that were apparently undergoing longitudinal fission were occasionally observed (Fig. 1c).

Sieve elements of palms infected with the flagellates contained few plastids in contrast to those that did not contain the organisms. The plasmalemma of infected sieve elements appeared to be intact but an amorphous electron-opaque material was frequently present between the cell wall and plasmalemma of the infected elements. Although the diameter of sieve-plate pores in sieve tubes of young inflorescences and leaves of coconut palm was 0.3 to 1.0 μm, the flagellates were apparently able to pass through sieve-plate pores and promote progressive infection.

On the basis of our preliminary study alone it is obviously not possible to determine the relation between *Phytomonas* and the coconut palm. We realize that the presence of the flagellates may be the result rather than the cause of the hartrot disease and that the causal relationships need to be proved according to Koch's postulates. However, the absence of organisms other than flagellates in the earliest symptom of the disease and the correlated increase and spread of the flagellates in sieve tubes as the disease progresses suggest that the flagellates are pathogenic to their hosts and hence the possible causative agents of hartrot. The distinct correlation between the presence of the flagellates in sieve tubes and the wilt of coffee plants in

Fig. 1. Electronmicrographs of the trypanosomatid flagellate *Phytomonas* in the phloem of young inflorescences of coconut palms affected by hartrot. (a) Transverse section of a differentiating vascular bundle in a palm that had early symptoms of the disease. Arrows indicate recently matured sieve elements filled with the flagellates; *M*, immature metaxylem; *S*, immature sieve elements (scale bar, 10 μm). (b) Transverse section of the phloem in a palm that had advanced symptoms of the disease, showing *C*, companion cell; *F*, fiber; *P*, phloem parenchyma cell; *S*, sieve elements free of flagellates (scale bar, 5 μm). (c) Transverse section of a flagellate that was apparently undergoing longitudinal fission (scale bar, 0.5 μm). (d) Longitudinal section of a sieve element filled with the flagellates. Arrows indicate the DNA portion of kinetoplasts (scale bar, 1 μm). (e) Similar to (b) but more magnified; *C*, companion cell; *P*, parenchyma cell (scale bar, 2 μm).

Suriname (*7*) further supports the idea that *Phytomonas* may be pathogenic to its plant hosts if it infects the food-conducting tissue. It is possible that infection of the flagellates in laticifers is not injurious or is less injurious to the host plants since laticifers are mainly excretory structures. It is of interest that the two reports of *Phytomonas* infection in nonlaticiferous plants are both from Suriname, and are both associated with a disease. Vectors of the trypanosomatids that infect the phloem of coffee and the coconut are yet to be determined.

Even if *Phytomonas* is proved to be nonpathogenic to coconut palms, their presence in sieve tubes is of biological significance. The flagellates can thrive under three apparently different environmental conditions. In addition to their insect hosts, they thrive in laticifers that chiefly contain particles belonging to the hydrocarbon familes of terpenes (*16*) and in sieve tubes that contain mostly sugars.

Although some aspects of the relationship of *Phytomonas* to its laticiferous plant host have recently been studied (*2*, *6*, *13*, *17*), little is known about the relation of the flagellate to its nonlaticiferous plant host. Further study of the biology of this curious trypanosomatid is essential to prove its apparent pathogenicity to some plants.

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8. Sieve tubes are the main conduit for long-distance transport of material in the food-conducting tissue, the phloem, of higher vascular plants. A sieve tube is comprised of sieve-tube elements disposed end to end in a long series. Sieve element is a comprehensive term which includes both sieve-tube elements and the main conducting unit of phloem in lower vascular plants—the sieve cells. Companion cells are specialized parenchyma cells that are normally associated with sieve-tube elements but are not directly involved in long-distance transport of material. Functional sieve elements are typically enucleate but contain a thin parietal cytoplasm. For more details of phloem structure in palms → M. V. Parthasarathy [*Am. J. Bot.* **55**, 1140 (1968); *Protoplasma* **74**, 265 (1974)].
9. "Hartrot" was first reported in Suriname in 1906. It has also been called lethal yellowing, bronze-leaf wilt, Coronie wilt, and "unknown disease." It exhibits many but not all the symptoms of lethal yellowing that has destroyed thousands of coconut palms in the Caribbean Islands, West Africa, and southern Florida. Mycoplasma-like organisms are suspected of being the causative agents of lethal yellowing. Mycoplasmas are highly pleomorphic prokaryotes (cells that lack membrane-bounded nucleus) that contain DNA and ribosomal RNA. They are bounded by a single unit membrane, and, in contrast to bacteria, do not have a rigid wall. Also, mycoplasmas are highly resistant or insensitive to penicillin but are inhibited by tetracyclines. The coconut cultivar "Malayan Dwarf" is resistant to lethal yellowing. On the other hand, none of the coconut varieties seem to be resistant to the hartrot.
10. More than 120 vascular bundles were examined from randomly chosen blocks that contained samples of material from palms in advanced stages of the disease.
11. A. Lafont, *Bull. Soc. Pathol. Exot.* **4**, 467 (1911).
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14. → J. J. Paulin and R. B. McGhee, *J. Parasitol.* **57**, 1279 (1971).
15. Kinetoplast is a DNA-rich organelle and is associated with mitochondrion. Members of the order Kinetoplastida are characterized by the possession of the kinetoplast.
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18. We thank Dr. R. Barclay McGhee, University of Georgia, Athens, for encouragement and for confirming our identification of the organisms.

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Leeuwenhoek's Observation of Bacteria

Abstract. *A form of dark-field illumination was produced that allows bacteria in aqueous suspension to be observed with both a Leeuwenhoek microscope and a compound microscope without a condenser. Possibly, this illumination was Leeuwenhoek's "particular method of observing" bacteria.*

Three hundred years ago Antonie van Leeuwenhoek used his single-lens microscope to discover the existence of bacteria and protozoa; he reported this in his 18th letter to the Royal Society of London (*1*). However, he did not disclose his method of observation because, as he stated, his "particular method of observing" he "kept for himself alone." In other words, the key to his success was not the use of a unique microscope, but a special method of observation that provided the required contrast between the cells and the suspending fluid. It would appear that, from Leeuwenhoek's time to the present, no one has been able to deduce and duplicate his method of observation. Dobell (*1*) concluded that Leeuwenhoek must have discovered some simple method of obtaining dark-field illumination; in fact, Leeuwenhoek's description of red blood cells appears to verify this. He described them as looking like "sand grains that one might bestow upon a piece of black taffety silk" (*1*).

I have examined several ways for obtaining dark-field illumination at low magnifications—methods that would have been simple enough for Leeuwenhoek to have used. The following is a description of a procedure that proved successful with: (i) a Leeuwenhoek microscope copy with approximately 100-fold magnification; (ii) a 30-power modern microscope ocular used in reverse fashion as a magnifier; (iii) a modern 32-power objective used as a magnifier; and (iv) a Leitz Orthoplan microscope set for 100- or 250-fold magnifications and with its condenser removed. Leeuwenhoek's microscopes magnified up to approximately 300 diameters. He is known to have used capillaries and paired thin glass plates for

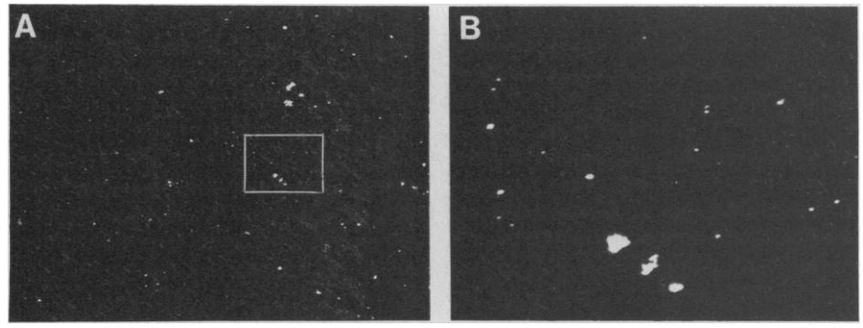

Fig. 1. *Escherichia coli* individual cells, paired cells, and cell clumps as viewed with combination of a ×10 objective and a ×10 eyepiece. (A) Total magnification of cells is ×57. (B) Total magnification of cells is ×283 for small area of (A) just right of center.