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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7. G. Stahel, *Phytopathol. Z.* **4**, 65 (1931); *ibid.* **5**, 539 (1932); *ibid.* **6**, 335 (1933). Stahel's observations were confirmed by later works of J. H. van Emden [*Meded. Landbouwhogesch. Opzoekingsstn. Staat Gent.* **27**, 776 (1962)], and H. Vermeulen [*J. Protozool.* **10**, 216 (1963)].
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 see 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.
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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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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

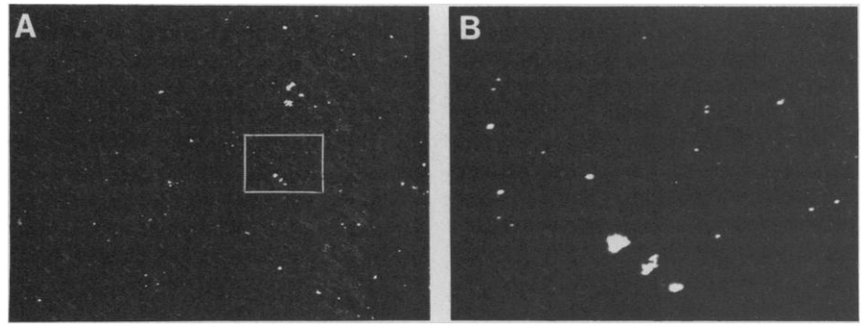

Fig. 1. *Escherichia coli* individual cells, paired cells, and cell clumps as viewed with combination of a ×10 objective and a ×10 eyepiece. (A) Total magnification of cells is ×57. (B) Total magnification of cells is ×283 for small area of (A) just right of center.

examination of fluids. My experiments with capillaries showed optical aberrations too great for clear resolution of bacteria, but a wet mount with paired cover slips or a cover slip and slide was acceptable. I used a simple Bausch and Lomb Nicholas spotlight illuminator as the light source; Leeuwenhoek used sunlight or a candle, sometimes in conjunction with magnifying mirrors to concentrate and direct the light. For the Leitz Orthoplan, I placed the light source beneath the front of the microscope stage with the light aimed at the sample. The light beam was at an angle of 45° with the plane of the sample (135° with the optical axis of the microscope). Suspensions of *Escherichia coli*, *Arthrobacter globiformis*, and nonsporulated *Bacillus megaterium* were examined in this manner. At 100- and 250-fold magnifications, the cells were easily seen (Fig. 1) because they were brightly illuminated against a black background as in dark-field illumination. This was true for cells in suspensions as well as those attached to the glass. Similar lighting was used for the Leeuwenhoek microscope and the other magnifiers, and it was found that dark-field illumination occurred at lighting angles of 45° or less, but that illumination of the cells decreased correspondingly as the angle was decreased from this value. Fungal mycelium (*Mucor* species) was clearly seen as dark-field with all magnifiers, including the Leeuwenhoek scope. A bacterium, *Bacillus mycoides*, that had not sporulated was observed as dark-field with the Leeuwenhoek microscope; the cells were seen coming into and out of the focal plane as they flowed past the field being viewed. This observation, including the appearance of the cells, was checked by observing the preparation with a phase microscope.

For Leeuwenhoek to have used this procedure would merely have required a beam of sunlight or candlelight striking his sample from behind at an angle of 45°. He may, however, have concentrated and aimed the beam with his magnifying mirrors. In addition to this, I suggest that he may have used his microscopes of lower power—that is, those magnifying in the range of 100 to 150 diameters—to observe suspended bacteria. These would have provided him with a greater depth of field for obtaining focus as compared to his instruments of greater magnifying power.

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Ant-Aphid Association: Role of Aphid Alarm Pheromone

Abstract. *When attacked by predators, aphids secrete alarm pheromones that cause nearby aphids to disperse. Ant-associated (myrmecophilous) aphid species disperse less readily than nonmyrmecophilous species. Ant exposure further depresses the dispersive alarm behavior of myrmecophilous species. The ant* Formica subsericea *responds to aphid alarm pheromone in a way that is beneficial to the aphid. These findings support our hypothesis that myrmecophilous aphids depend more on ants for protection from predators than on their own dispersive powers.*

Aphids are small, soft-bodied insects that feed on plants. Their sedentary, gregarious habits make them particularly vulnerable to predator attack. Aphids have countered this threat by evolving a dual, self-serving and altruistic defensive system involving the aphid's cornicle secretion. When attacked by predators, aphids secrete cornicle droplets (Fig. 1). These "sticky" droplets, composed largely of triglycerides, can impede an attacking predator and result in the release of aphid prey (*1*). The droplets also contain hydrocarbons, notably *trans*-β-farnesene, which serve as alarm pheromones that inform nearby aphids of impending danger. Aphids then fall, jump, or walk away to escape (*2*).

Certain aphid species are also protected from predators by the ant species that tend them (*3*). In field studies, we found that ant-associated (myrmecophilous) aphids disperse less readily to alarm pheromones than do nonmyrmecophilous species. We suspected that myrmecophilous aphids may depend more on ants for protection than on their own dispersive powers. We tested this hypothesis under controlled conditions in the laboratory and found a marked dif-

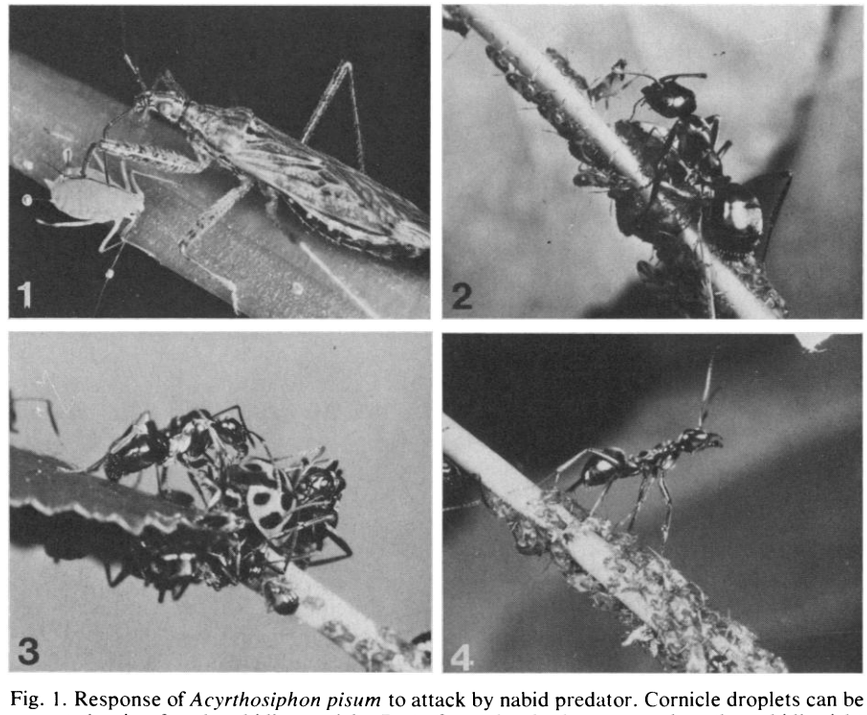

Fig. 1. Response of *Acyrthosiphon pisum* to attack by nabid predator. Cornicle droplets can be seen at the tip of each aphid's cornicle. Part of one droplet has smeared on the aphid's right antenna. Fig. 2. *Chaitophorus populicola* standing in response to antennation by *Formica subsericea*. Continued antennation results in excretion of a honeydew droplet that is collected by the ant. Fig. 3. Ants are shown attacking a coccinellid aphid predator by biting with mandibles and smearing contents of Dufour's gland from abdominal tip. Fig. 4. An ant responds to *trans*-β-farnesene by raising and extending antennae, opening mandibles, and orienting toward pheromone source.