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TRYPANOSOMATID PROTOZOA IN FRUIT OF SOLANACEAE IN SOUTHEASTERN BRAZIL

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Fruits of cultivated and indigenous Solanaceae from Southeastern Brazil have been examined for the presence of trypanosomatid flagellates. The 14 species found infected were: Capsicum annuum, C. praetermissum, Lycopersicon esculentum, Nicandra physaloides, Physalis angulata, Solanum sp., S. americanum, S. concinnum, S. diflorum, S. erianthum, S. gilo, S. robustum, S. variabile *and* S. viarum. *The pentatomid hemipteran* Arvelius albopunctatus *experimentally transmitted flagellates to fruits of some species. Cultures of flagellates were obtained from fruits of eight species of Solanaceae and from* A. albopunctatus.

Key words: Phytomonas – transmission – insect vectors

Trypanosomatid flagellates (Protozoa, Kinetoplastida) of the genus *Phytomonas* are associated to devastating diseases of coffee (Stahel, 1931), cassava (Kitajima et al., 1986), coconut (Parthasarathy et al., 1976) and oil palms (Dollet et al., 1977), being probably transmitted to their plant hosts by phytophagous insects, particularly hemipterans (Dollet, 1984; Camargo et al., 1990).

Flagellates are also known to occur in laticiferous plants (Dollet, 1984) and in edible fruits of various plant families (Conchon et al., 1989), including Solanaceae of economical importance such as *Lycopersicon esculentum,* tomato (Gibbs, 1957).

While the evidence for the pathogenicity of these flagellates in coffee, palms and cassava is substantital, nothing is known about their pathogenic role in fruits what, however, is not equivalent to saying that there is none. Here we report on the occurrence of flagellates in fruits of 14 out of 17 species of Solanaceae in southeastern Brazil. This finding, beside enlarging our knowledge on fruit trypanosomatids, may have considerable practical importance if these flagellates are eventually found to be plant pathogens.

MATERIALS AND METHODS

Surveying of Solanaceae – Vegetative parts and fruits of indigenous and cultivated Solanaceae were collected in São Paulo State, southeastern Brazil. Backyard gardens or farm fields in the localities of Campinas, Pindamonhangaba, Pirassununga, and also nature reserves of São Paulo City (Cidade Universitária, Pacaembu and Parque Raposo Tavares) were most intensively explored.

Sap of roots, stems and fruits of the collected plants were examined by phase contrast microscopy for the presence of flagellates. Smears fixed in methanol and stained with Giemsa were used for morphological studies and drawings by Camera lucida.

Solanaceae were identified by Dr. L. Rossi from the Instituto de Botânica de São Paulo whereas insects collected on Solanaceae were identified by Dr J. Grazia from the Departamento de Zoologia da Universidade Federal do Rio Grande do Sul, Brazil.

Cultures – Sap of positive fruits and salivary glands of *A. albopunctatus* were inoculated in tubes containing biphasic medium made of 3 ml of 2% agar base and rabbit blood plus 3 ml

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overlay of complex nutrient medium (Roitman et al., 1972) containing 250 μg/ml of ampicillin, 250 μg/ml of gentamicin and 1.25 μg/ml mycostatin. Cultures cleaned of contaminants were thereafter kept at 25 °C in antibiotic-free biphasic medium.

Experimental infections – Flagellate-free tomatoes were inoculated with culture flagellates isolated either from fruits or salivary glands of insects, as previously described (Conchon et al., 1989). Attempts to infect tomatoes were also done by exposing flagellate-free tomatoes to adults of *Arvelius albopunctatus* collected on naturally infected fruits of *Solanum viarum*. Fruits of other Solanaceae used in similar infection experiments were collected in areas ascertained to be free of the flagellose by exhaustive microscopic examination of sample specimens.

Nymphs of *A. albopunctatus*, raised from eggs in the laboratory and grown on clean tomatoes, were fed on tomatoes inoculated with cultures of flagellates isolated from *S. variabile* and *A. albopunctatus*. These insects where then fed on clean tomatoes to verify ability to transmit the infection.

Histology – Blocks of about 1 cm^3 of tomatoes naturally or experimentally infected by the bites of *A. albopunctatus* were cut off and fixed in 2% glutaraldehyde, dehydrated in ethanol series, embedded in glycolmethylacrylate plastic (Historesin, LKB), sectioned at 2-4 μm and stained with 0.05% toluidine blue for light microscopy examination.

RESULTS AND DISCUSSION

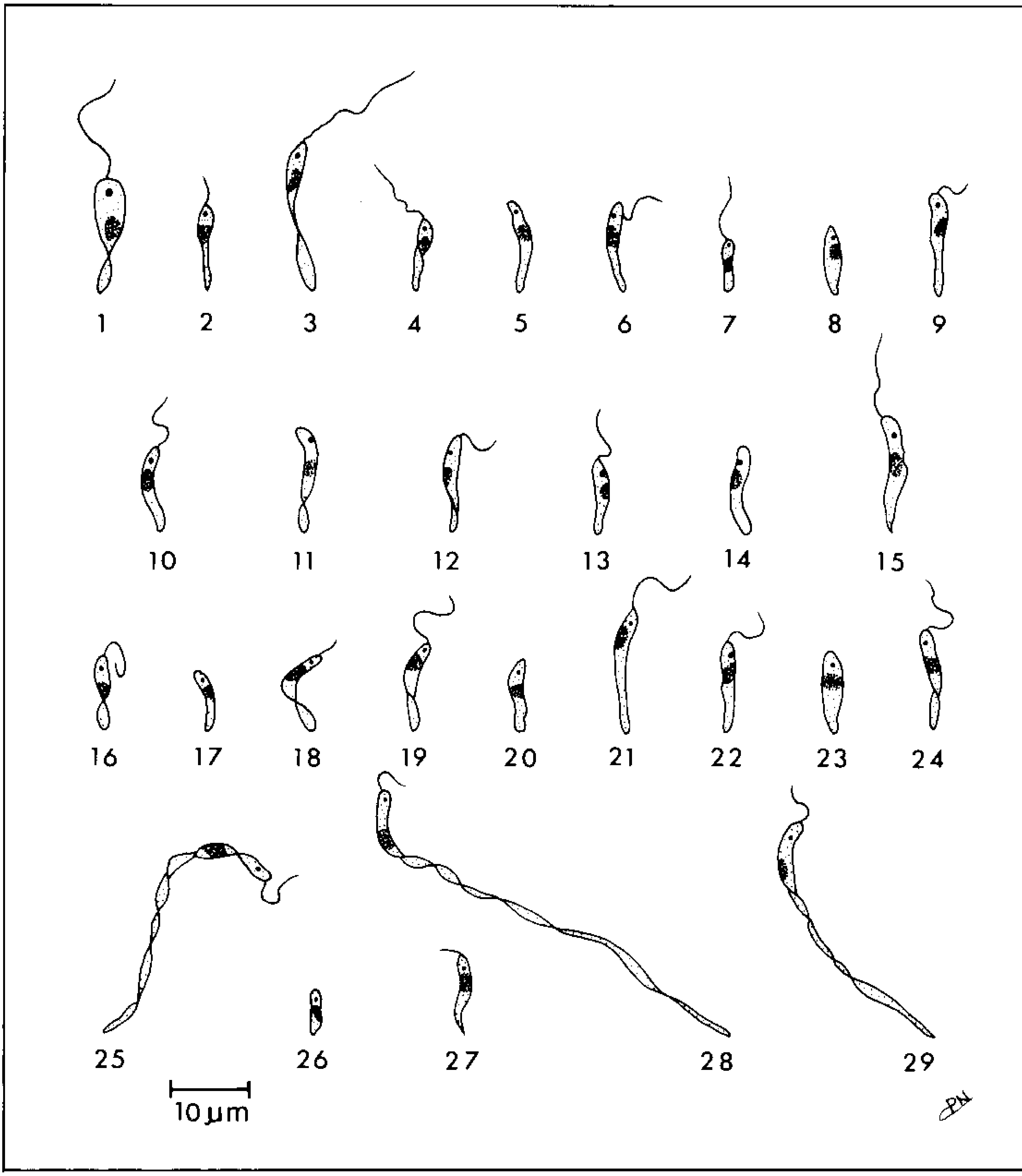
Occurrence – Fruits of the following species of cultivated Solanaceae, collected in backyard gardens, were found infected with flagellates: *L. esculentum* (tomato, variety "cherry"), *S. gilo* (jiló) and *Capsicum annuun* (red pepper). Of these, only the first two species were known to harbor trypanosomatids (Gibbs, 1957; Fiorini et al., 1986). Among Solanaceae collected on the wild, fruits of the following were positive for flagellates: *C. praetermissum, Nicandra physaloides, Physalis angulata, Solanum* sp., *S. americanum, S. concinnum, S. diflorum, S. erianthum, S. robustum, S. variabile* and *S. viarum*. None of those had been reported before as hosts of trypanosomatids. Not all plants of a given plantation or shrub were infected and the rate of infection among fruits of a certain plant ranged from 2 to 65%. In certain areas, plants were consistently free of parasites. The following Solanaceae were never found infected: *Physalis sp.* and *S. ciliatum* in addition to a still unidentified species.

The fruits of the evergreen perennials *S. variabile* and *S. robustum* were found infected through all seasons. In short-lived or deciduous Solanaceae, such as *S. viarum* and *N. physaloides*, flagellates were found only at the end of summer. Fruits of cultivated tomato and red pepper were found infected in autumn/winter near *S. viarum* with overripe and decaying fruits.

Transmission – Solanaceous plants with parasitized fruits have frequently been found occupied by nymphs and adults of the pentatomid hemipteran *A. albopunctatus*, which also presented flagellates in their digestive tube and salivary glands. *Phthia picta*, a coreid hemipteran, was found associated with jiló.

The close association between these phytophagous bugs and Solanaceae made them suspect of being flagellate vectors. Under experimental conditions it was indeed possible to infect fruits through the biting of infected bugs. Tomatoes (15 out of 18), peppers (5 out of 6), fruits of *S. erianthum* (4 out of 12) and of *S. viarum* (3 out of 8) became infected after one week of exposure to adults of *A. albopunctatus* collected in the field on infected fruits of *S. viarum*. Fruits of *S. gilo* remained negative after such exposure.

Flagellate-free tomatoes also became infected when artificially inoculated with flagellates from a culture originary from the salivary glands of *A. albopunctatus*. Moreover, when these so infected tomatoes were used to feed laboratory-raised nymphs of the bug, 8 out of 30 insects became positive, presenting flagellates in their salivary glands. Tomatoes also became infected when artificially inoculated with flagellates of a culture originary from *S. variabile* and thereafter transmitted the infection to 2 out of 20 *A. albopunctatus* fed upon them. Experimentally infected insects thereafter transmitted the acquired flagellates to clean tomatoes. These facts strongly suggest that the phytophagous pentatomid *A. albopunctatus*, although not exclusively, may be a vector of



Flagellates of Solanaceae fruits and insects. 1-3: from *Nicandra physaloides.* 4-6: from *Solanum americanum.* 7-9: from *S. concinnum.* 10-12: from *S.* sp. 13-15: from *S. viarum.* 16-18: from *S. erianthum.* 19-21: from *S. robustum.* 22-24: from *S. variabile.* 25-29: from salivary glands of *Arvelius albopunctatus.*

flagellates of Solanaceae. It is also known that the coreid *P. picta* transmits the flagellates to tomatoes (Jankevicius et al., 1989). However, the ubiquity of *A. albopunctatus* renders this insect a more probable candidate for transmitting flagellates among different species of Solanaceae.

Histology – Fruits were the only plant parts found infected. Flagellates could be seen nowhere in roots, stems or leaves. In fruits, flagellates were located under the skin immediately subjacent to tiny discolored spots corresponding to insect's bite. In a few cases, flagellates were also seen in the juicy centers of the fruits. In histological preparations of tomatoes experimentally infected through insect bite, trypanosomatids could be detected as clusters in the intercellular space or inside parenchymal cells at superficial locations.

Flagellates – Flagellates in fruits were never abundant. Oftenly, no more than a few parasites could be seen in fruits' sap. They were relatively short (body length 10-15 μm) and predominantly of the promastigote type sometimes presenting a few torsions along their longitudinal axis (Fig.). A few forms without an apparent flagellum were also present. The kinetoplast, diagnostic of kinetoplastida, was present in all forms. There were no significant morphological differences between flagellates originating from the various Solanaceae. Flagellates in fruits experimentally infected – either through insect bites or culture inoculation – were more abundant but morphologically similar to flagellates from naturally infected fruits.

Flagellates from salivary glands of *Arvelius* were also promastigotes but in general larger than flagellates from fruit (body length up to 50 μm) presenting several torsions along their bodies (Fig.). The considerable morphological differences between insect and fruit flagellates may make it objectionable to consider these forms as deriving from the same species of trypanosomatid. However, it is known that the tomato parasite, *Phytomonas serpens*, exhibits considerable polymorphism in its life cycle where large, twisted promastigotes from insect salivary glands give rise in fruits to smaller and simpler forms similar to those reported here (Jankevicius et al., 1989). Therefore, it seems reasonable to assume, also in the present case, that insect and fruit flagellates may represent developmental stages of the same trypanosomatid species, moreover since fruits experimentally infected through insect bites yielded flagellates similar to those of natural infections.

A total of 16 cultures were obtained from fruits of eight different species (*N. physaloides*, 1; *Solanum* sp., 1; *S. americanum*, 1; *S. concinnum*, 1; *S. erianthum*, 1; *S. robustum*, 2; *S. variabile*, 4; *S. viarum*, 5) plus two cultures from *Arvelius* salivary glands. Flagellates from cultures were of the promastigote type, some with torsions along their bodies, a trait common to flagellates of the genus *Phytomonas*.

Flagellates either from the various fruits or fruit cultures were morphologically indistinguishable. This is not tantamount to saying that they are the same species of trypanosomatid. Morphology and host origin help little in telling apart species of trypanosomatids (Wallace et al., 1983; Sbravate et al., 1989). Therefore, the available data do not permit to ascertain whether we are facing one or a few flagellate species circulating among Solanaceae fruits through the vectoring of one or more phytophagous insects.

As to the genus of these flagellates, it would be standard procedure to place them under *Phytomonas*, since this genus, by definition, harbors trypanosomatids of plants. However, it has been recently shown that trypanosomatids of other genera may also be recovered from fruits (Conchon et al., 1989). Therefore, the taxonomic placement of the trypanosomatids from Solanaceae has to wait for the biochemical and serological analysis of the cultures as recommended by Wallace et al. (1983).

Unfortunately we are not prepared to assess the pathogenicity of these flagellates in fruits. However, the unequivocal pathological role of trypanosomatids to many other plants should serve as an alert to the risk posed by these flagellates to solanaceous crops.

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