Identification of a Rhabdovirus in Soursop (*Annona muricata*)

E. W. KITAJIMA and CLAUDIA RENATA F. MARTINS, Departmento de Biologia Celular, Universidade de Brasília, 70919 Brasília, DF; and ANTONIO A. SANTOS, Empresa de Pesquisa Agropecuaria do Ceara (EPACE), Av. Rui Barbosa 1246, 60000 Fortaleza, CE, Brazil

ABSTRACT

Kitajima, E. W., Martins, C. R. F., and Santos, A. A. 1993. Identification of a rhabdovirus in soursop (*Annona muricata*). Plant Dis. 77:276-278.

Soursop (*Annona muricata*) plants with yellow blotches on the leaves were found in a small experimental orchard of Empresa de Pesquisa Agropecuaria do Ceara, at Pacajus, state of Ceara. Symptoms were reproduced by mechanically inoculating soursop seedlings and by grafting on soursop, sweetsop (*A. squamosa*), and biriba (*Rollinia deliciosa*). No seed transmission was observed. Rhabdoviruslike particles were consistently found in leaf-dip preparations from the diseased soursop plants collected in the field and from experimentally infected soursop, sweetsop, and biriba. In thin sections of leaf tissues from naturally or experimentally infected plants, particles were found in the cytoplasm within membrane-bound cavities. Based on these results, we conclude that yellow blotch of soursop is caused by a rhabdovirus unrelated serologically to maize mosaic, lettuce necrotic yellows, and sow thistle yellow vein viruses.

Additional keywords: soursop yellow blotch virus

Soursop (*Annona muricata* L.), also known as guanabana, is an Annonaceae fruit tree native to the American tropics. In Brazil, it is primarily cultivated in the northeast on small plantations. It produces large, spiny, green-skinned fruits up to 4 kg each, with whitish, aromatic pulp. They are used for juice and in the ice cream industry. Although soursop is a minor cultivated tropical fruit, it is thought to have great potential.

Yellow blotches were found on the leaves of soursop trees in a small experimental orchard at the Experimental Station of Empresa de Pesquisa Agropecuaria do Ceara (EPACE), at Pacajus, state of Ceara (Fig. 1). Affected plants were slightly less developed than were those without symptoms. This orchard had 152 trees, and in the initial visit in August 1988, 36 (24%) showed symptoms.

In this paper, we provide evidence that this yellow blotch of soursop is caused by a rhabdovirus.

MATERIALS AND METHODS

Leaves and twigs were collected and taken to Brasília for further study, and the orchard was kept under observation to investigate possible dissemination of the disease.

Extracts from leaves with yellow blotch symptoms were prepared by grinding the leaves with 0.01 M, pH 7 phosphate buffer and 0.1% sodium sulfite. The extracts were applied to leaves of soursop, sweetsop (*A. squamosa* L.), biriba (*Rollinia deliciosa* Saff.), and the following test plants: *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., *Gomphrena globosa* L., *Nicotiana tabacum* L., *N. glutinosa* L., *N. rustica* L., *N. benthamiana* Domin., *Datura stramonium* L., *Nicandra physalodes* (L.) Gaertn., *Lycopersicon esculentum* Mill., *Capsicum annuum* L., *Lactuca sativa* L., *Brassica oleracea* L. var. *acephala* DC., *Phaseolus vulgaris* L., and *Vigna unguiculata* (L.) Walp. Twigs from affected soursop plants were also fork grafted onto 50 soursop, 10 sweetsop, and 10 biriba plants, all 7–8 mo old.

Leaf-dip preparations from naturally or experimentally infected soursop, sweetsop, and biriba plants were obtained by mincing leaf tissue directly into 1% aqueous sodium silicotungstate. These tissue samples were also processed for ultrathin sections by fixing them in a mixture of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M, pH 7.2, cacodylate buffer, followed by postfixation in 1% osmium tetroxide, en bloc staining with 0.5% aqueous uranyl acetate, dehydration in acetone, and embedding in Spurr's medium. Thin sections were produced in an LKB Ultrotome III microtome equipped with an RCM diamond knife, then mounted on copper grids and stained with uranyl acetate and lead citrate. In situ immunogold labeling experiments with specific antisera and protein A-conjugated colloidal gold were also performed in sections of tissues fixed only in the glutaraldehyde-paraformaldehyde mixture and embedded in LRGold at −20 C (5). We used antibodies against the following plant rhabdoviruses: maize mosaic (produced by us), lettuce necrotic yellows, and sow thistle yellow vein. Examinations were made in a JEOL JEM 100C electron microscope.

Serological tests using these antibodies were also made by agar gel double-diffusion, with leaf extracts from healthy and diseased soursop plants as antigens.

RESULTS AND DISCUSSION

The virus was mechanically transmitted to only about 20% (28 of 138) of the soursop seedlings inoculated at the four-leaf stage, 3 wk after emergence. Vein-clearing and leaf-distortion symptoms were evident 3–4 wk after inoculation. Infected plants became stunted and stopped growing, but rarely died. In another experiment, only three of 35 1-yr-old, mechanically inoculated soursop plants became infected. On the other hand, no transmission was observed with the 20 biriba seedlings or the 7–8-mo-old biriba and sweetsop plants (10 each).

Fig. 1. Yellow blotch symptoms on soursop (*Annona muricata*) leaf.

This work received financial support from Conselho Nacional de Desenvolvimento Cientifico and Tecnologico and Programa de Apoio ao Desenvolvimento Cientifico e Tecnologico.

Accepted for publication 25 September 1992.

None of the inoculated herbaceous test plants developed symptoms.

Yellow blotch symptoms could be easily transmitted by grafting from soursop to soursop. The original symptoms appeared in young shoots about 1–1.5 mo after the grafting. When the graft took, leaves initially showed vein clearing, which developed into yellow patches as the leaves matured. Leaf symptoms developed in the same way on new branches. Sweetsop and biriba also developed vein clearing after fork grafting from soursop with yellow blotch, but only in the leaves of shoots that grew near the grafting site. The causal agent may not have moved from the inoculation site. The yellow blotch agent was not detected in 480 seedlings grown from the seed of the affected soursop plants.

Leaf-dip preparations from soursop plants with yellow blotch symptoms consistently contained rhabdoviruslike particles, but preparations from the symptomless plants did not. These particles were also present in soursop infected either by mechanical inoculation or by grafting, as well as in sweetsop and biriba plants infected by grafting. None of the herbaceous plants mechanically inoculated with extracts from affected soursop plants contained these particles. The particles averaged 60–70 nm by 250–300 nm and contained a 40–50-nm-wide inner component with a transverse periodicity of 5 nm (Fig. 2).

Examination of thin sections of leaf tissue from yellow blotch-affected soursop, sweetsop, and biriba revealed a large number of rhabdoviruslike particles in membrane-bound cytoplasmic cavities of the endoplasmic reticulum, but not in the perinuclear space (Fig. 3A,B). These particles were about 60 nm wide by 400 nm long, although occasionally particles up to 700 nm long, interpreted as dimers, could be seen (Fig. 3B). Frequently, masses of wavy filaments (Fig. 3B), similar to previously described viroplasms, were found in the cytoplasm adjacent to the group of particles. These wavy filaments might represent viral nucleocapsids prior to the budding by which they acquire the outer envelope. In some instances, a clear continuity between the membranes of the endoplasmic reticulum and of the rhabdoviruslike particles could be seen (Fig. 3C), and we interpret this continuity as an intermediate stage in the budding process.

Immunogold labeling experiments performed with sections of LRGold-embedded tissues did not reveal specific labeling with antibodies against lettuce necrotic yellows, sow thistle yellow vein, or maize mosaic rhabdovirus. Similar results were obtained in agar gel double-diffusion tests. Therefore, we believe the virus is not identical to any of these rhabdoviruses.

Preliminary attempts to purify this rhabdovirus following protocols described by Jackson et al (3) have not been successful.

Unfortunately, the original orchard was destroyed in 1990 when the Experimental Station was closed. At the last count in August 1990, the number of affected plants had increased to 48%. This rise from 24% in 1988 indicates the spread of the disease, probably by a yet-to-be-identified insect. Yellow blotch of

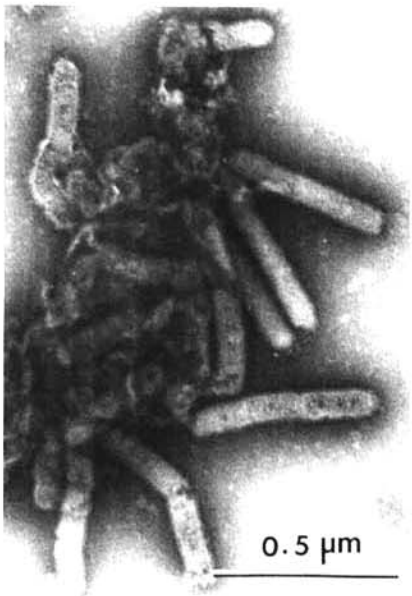

Fig. 2. Rhabdoviruslike particles in a negatively stained leaf-dip preparation from field soursop plants exhibiting yellow blotches on the leaves.

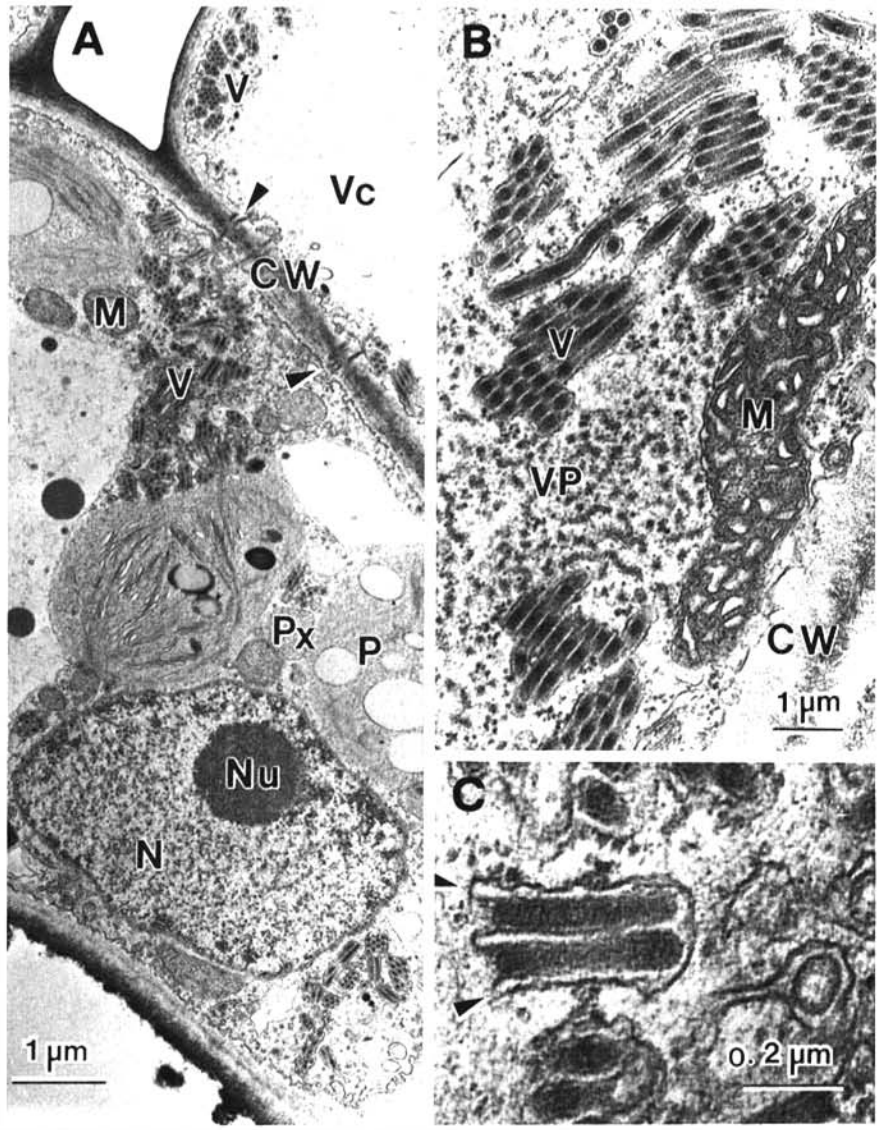

Fig. 3. Electron micrographs of thin sections from leaf parenchyma of soursop experimentally infected with soursop yellow blotch virus (SYBV). **(A)** Low-magnification view of spongy parenchyma cells containing a large number of bacilliform particles (V) in the cytoplasm. The nucleus (N) appears normal, but chloroplasts (P) are disorganized. **(B)** Detail of rhabdoviruslike particles (V) within membrane-bound cavities. The adjacent cytoplasm (VP) contains wavy filaments, interpreted as naked nucleocapsids forming a viroplasm. **(C)** Detail of two rhabdoviruslike particles budding into the endoplasmic reticulum cisterna. Arrows show continuities of the particles's outer envelopes with the membrane of the endoplasmic reticulum. CW = cell wall (arrows = plasmodesmata), M = mitochondrion, Nu = nucleolus, Px = peroxisome, and Vc = vacuole.

sours was also recently detected at low incidence by one of us (A. A. S.) in two other localities in the state of Ceara (Barreiras and Cascavel), which indicates that the disease is spreading in the state.

The evidence obtained so far suggests that this soursop disease is caused by a rhabdovirus, which we are provisionally designating soursop yellow blotch virus (SYBV). Since the particles accumulate in the endoplasmic reticulum rather than in the perinuclear space, SYBV should be included in type I of the classification of rhabdoviruses (2). This seems to be the first report of a virus disease in members of Annonaceae, although many fungal diseases have been reported in species of this family (1). It is likely that the virus might cause yield losses, especially when the infection occurs in young plants. Field studies are needed to understand the epidemiology of yield losses due to SYBV. The taxonomic status of SYBV in relation to other rhabdoviruses (2,3), many of which occur in Brazil (4), will only be possible after further investigations at the molecular level.

LITERATURE CITED

1. Cook, A. A. 1975. Diseases of Tropical and Subtropical Fruits and Nuts. Hafner Press, New York.
2. Francki, R. I. B., Milne, R. G., and Hatta, T. 1985. An Atlas of Plant Viruses, vol. 1. CRC Press, Boca Raton, FL.
3. Jackson, A. O., Francki, R. I. B., and Zuidema, D. 1987. Biology, structure, and replication of plant rhabdoviruses. Pages 427-508 in: The Rhabdoviruses. R. W. Wagner, ed. Plenum Publishing Co., New York.
4. Kitajima, E. W., De Sa, P. B., Ritzinger, C. H. S. P., and Rodrigues, M. G. R. 1991. Deteccao de particulas do tipo rhabdovirus em algumas compostas, aboboreira e mamoeiro. Fitopatol. Bras. 16:141-144.
5. van Lent, J. W. M., Groenen, J. T. M., Klinge-Oode, E. C., Rohrmann, R., Zuidema, D., and Vlak, J. M. 1990. Localization of the 34kd polyhedral envelope protein in *Spodoptera frugiperda* cells infected with *Autographa californica* nuclear polyhedra virus. Arch. Virol. 111:103-114.