Association of Rickettsialike Bacteria With Plum Leaf Scald Disease

E. W. Kitajima, M. Bakarcic, and M. V. Fernandez-Valiela

Professor, Phytopathologist, and Director, respectively, Dept. Biol. Cel., IB, Univ. Brasília, 70000 Brasília, DF, Brazil; and Est. Exp. Delta del Paraná, INTA, Campana, Prov. Buenos Aires, Argentina.

ABSTRACT

Rickettsialike bacteria were consistently found in the xylem vessels of Japanese plum (*Prunus salicina*) affected by leaf scald, a disease confined to the delta of the Paraná River, Argentina. This observation, besides some of the pathological characteristics of the leaf scald disease, suggested that this plum abnormality might have a rickettsial etiology.

Phytopathology 65:476-479

Plum leaf scald (PLS) is a disease so far recognized only at the delta of the Paraná River, Argentina. Since it was first reported around 1935 (5), the disease slowly spread to the lower parts of the delta, where it is still confined. PLS has been causing severe losses in the culture of the Japanese plum (*Prunus salicina* Lindl.) the most intensively cultivated fruit crop in the region (5, 6). This disease also affects other *Prunus* species such as *P. domestica, P. cerasifera, P. insititia,* and *P. americana,* inducing symptoms similar to that of *P. salicina*. *Prunus*

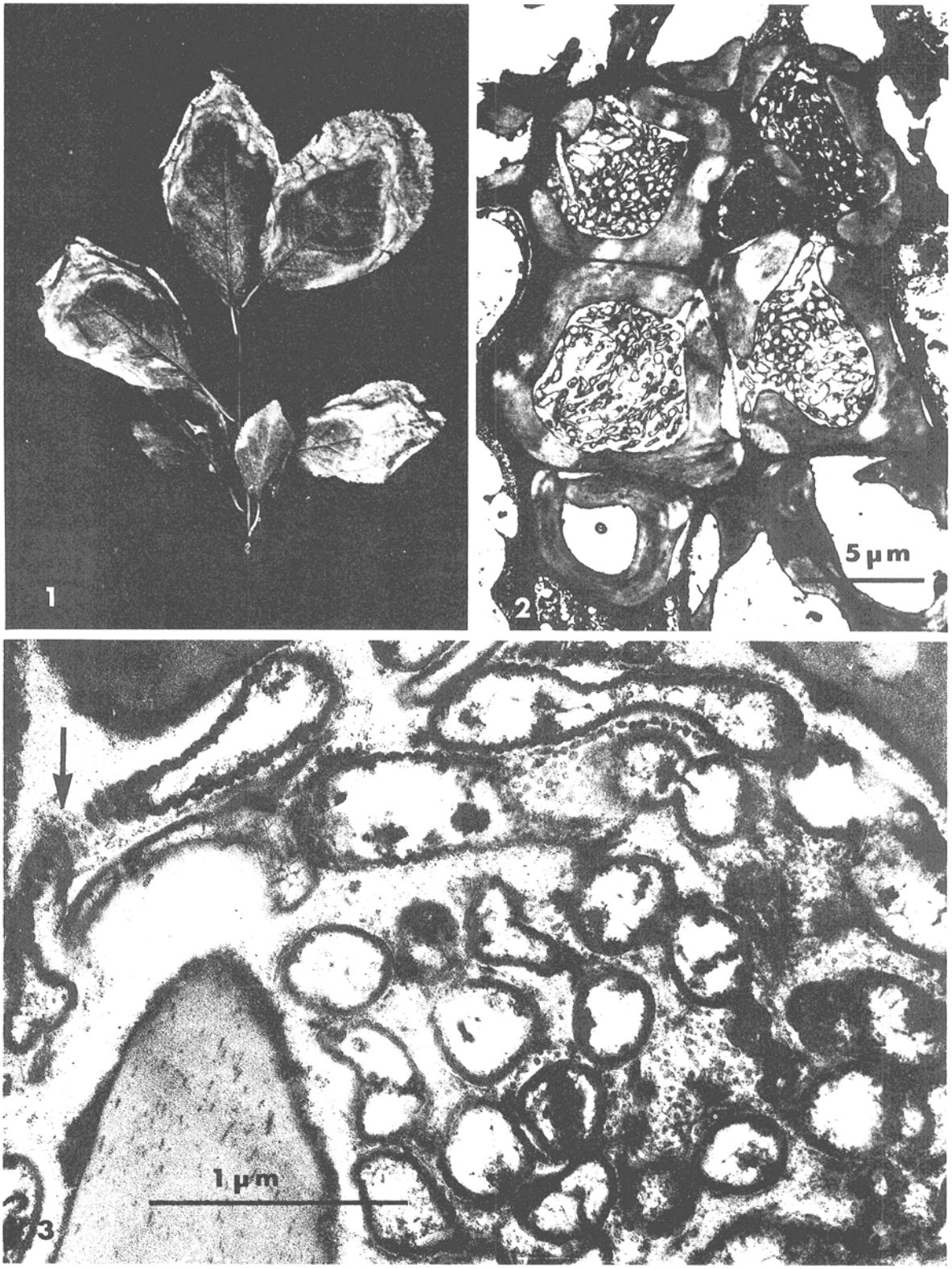

Fig. 1-3. 1) Leaves of Japanese plum showing typical symptoms of leaf scalding. (2-3) Electron micrographs of rickettsialike bacteria in the xylem vessels of PLS-affected plum leaf. A survey picture **2)** shows a group of tracheids invaded by the bacterioids. **3)** Shows detail of single cell, such as the thick and rippled cell wall. Arrow points to a tangential section of the bacterioid's cell wall.

amygdalus although susceptible to the PLS agent, does not show symptoms when invaded.

Symptoms usually appear at late December or early January. The first symptom is a slight and irregular chlorosis at the leaf border, which intensifies later. Finally, leaf margins become brown and dry, being separated from unaffected areas by a diffuse chlorotic band (Fig. 1). Plants with generalized leaf scalding appear as having been scorched. Upper branches become brittle, and in advanced phases of the disease, plants stop growing, and die within a few years. The fruits in highly susceptible cultivars do not reach normal size, and production is gradually reduced as the disease progresses (5, 6).

PLS is graft-transmissible and first symptoms appear in inoculated plants after 2 years. No microorganism or physiological conditions could be associated consistently with the disease, to which a viral nature was attributed (6). Insects are scarce on plum plants; a member of the family Flatidae, *Ormenis cestri* Berg., is one of the few present and may be a vector. The leaf scald agent is inactivated by hot-water treatment of living parts of the plum plant (2). The response of plum cultivars affected by PLS ranges from high susceptibility, to tolerance, or even immunity. Since natural hybridization is common, sources of resistance for control purposes are sought in such natural hybrids (3).

Some of the characteristics of PLS resemble those of diseases to which mycoplasmal or rickettsial etiology has been attributed recently (4, 7, 8, 9, 12-14). This led us to undertake an electron microscopical investigation on PLS-affected tissues. Details of the finding of a rickettsialike organism associated with PLS are presented in this communication.

MATERIALS AND METHODS.—Leaf materials were collected from eight plants of Japanese plum cultivar Gigaglia, which were graft-inoculated with PLS 2 years ago, and kept in insect-proof cages. Samples for electron microscopy were harvested at two different periods, from leaves exhibiting conspicuous chlorosis of the leaf margins. Small pieces (1 mm × 3 mm) of the chlorotic tissues, always including a veinlet, were fixed in a 3% solution of glutaraldehyde in 0.05 M phosphate buffer, pH 7.2, for 1-2 hours, rinsed thoroughly in 0.2 M buffer, immersed in which they were sent via air mail to Brazil, where post-fixation in OsO_4 and embedding in Epon was carried out. Thin-sections were stained with uranyl acetate and lead citrate, and examined in a Zeiss EM 9 electron microscope.

For control purposes, samples from four uninoculated, healthy plants were collected, processed, and examined as above described.

RESULTS.—Examination of the ultrathin sections from plum foliar tissues revealed that despite the long period and lack of refrigeration between aldehyde fixation and post-fixing in OsO_4, cells appeared reasonably preserved. In samples from PLS-affected plants, the most striking finding was the consistent presence of structures with typical bacterial features, in some bundles of xylem vessels (Figs. 2, 3). These bacterioids measured about 1-3 $\mu m \times 0.5$ μm, possessed a corrugated cell wall about 20-30 nm thick, and contained some dense granular material at the cell periphery and DNA-like fibrils in the central region (Fig. 3). No virus or mycoplasmalike organism was found in the parenchymatous or phloem tissues in PLS-affected plants. Bacterioids were never observed in tracheids from uninoculated control plum leaves.

Leaf samples from both diseased and healthy plants were also sent to the Department of Phytopathology of the Facultad de Agronomia, University of Buenos Aires, where attempts to isolate bacteria were made by E. A. Gotuzzo, with negative results.

DISCUSSION.—The size range and the corrugated cell wall of the bacterioids present in the xylem vessels of PLS-affected plum leaves are essentially similar to those found associated with some other plant disorders (7, 9, 12, 13, 14), which were considered analogous to rickettsia (1, 4). Since these rickettsialike organisms are consistently associated with PLS, they might represent the causal agent of this plum abnormality, rather than a virus.

The present finding, added to other known properties of PLS, indicates that this disease may probably be grouped together with Pierce's disease of grapevine (PDG), and peach phony disease (PPD), because these disorders share several common points, beside being associated with rickettsialike organisms, such as: (i) the possible agent occurs in the xylem vessels; (ii) the pathogen can be inactivated in vivo by hot-water treatment (2, 7, 11); and (iii) leaf scalding symptoms of PLS and PDG have the same pattern.

PDG agent is suceptible to antibiotic treatment (10), which is a strong argument favoring the rickettsial etiology of this disease. This point has yet to be demonstrated with PPD and PLS. Further detailed comparative works, particularly involving immunology, will certainly clear up this problem.

LITERATURE CITED

1. ANDERSON, D. R., H. E. HOPPS, M. F. BARILE, and B. C. BERNHEIM. 1965. Comparison of the ultrastructure of several rickettsiae, ornithosis virus, and Mycoplasma in tissue culture. J. Bact. 90:1387-1404.
2. BAKARCIC, M. 1972. Inactivación térmical del virus de la escaldadura de la hoja del ciruelo. Delta del Paraná 12 (13):61-69.
3. BAKARCIC, M., and M. A. DE SANTIS. 1969. Comportamiento a la escaldadura de la hoja de variedades locales de ciruelo. Delta del Paraná 9(12):35-42.
4. DAVIS, R. E., and R. F. WHITCOMB. 1971. Mycoplasmas, rickettsiae, and Chlamydiae: possible relation to yellows diseases and other disorders of plants and insects. Annu. Rev. Phytopathol. 9:119-154.
5. FERNANDEZ-VALIELA, M. V., and M. BAKARCIC. 1954. Nuevas enfermedades del ciruelo en el delta del Paraná, Argentina. Pages 2-6 *in* Inf. Invest. Agric. (IDIA) No. 84, Inst. Nac. Tecn. Agropec. (INTA), Buenos Aires, Argentina.
6. FERNANDEZ-VALIELA, M. V., and M. BAKARCIC. 1963. Escaldadura del borde de las hojas del ciruelo. Delta del Paraná 3(3):39-47.
7. GOHEEN, A. C., G. NYLAND, and S. K. LOWE. 1973. Association of a rickettsialike organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63:341-345.
8. HOPKINS, D. L., W. J. FRENCH, and H. H. MOLLENHAUER. 1973. Association of a rickettsialike bacterium with phony peach disease. Phytopathology 63:443 (Abstr.).

9. HOPKINS, D. L., and H. H. MOLLENHAUER. 1973. Rickettsialike bacterium associated with Pierce's disease of grapes. Science 179:298-300.
10. HOPKINS, D. L., and J. A. MORTENSEN. 1971. Suppression of Pierce's disease symptoms by tetracycline antibiotics. Plant Dis. Rep. 55:610-612.
11. HUTCHINS, L. M. 1933. Identification and control of the phony disease of peach. Ga. Off. State Entomol., Bull. 78. 55 p.
12. MARAMOROSCH, K., B. PLAVSIC-BANJAC, J. BIRD, and L. J. LIU. 1973. Electron microscopy of ratoon stunted sugar cane: microorganisms in xylem. Phytopathol. Z. 77:270-273.
13. NYLAND, G., A. C. GOHEEN, S. K. LOWE, and H. C. KIRKPATRICK. 1973. The ultrastructure of a rickettsialike organism from a peach tree affected with phony disease. Phytopathology 63:1275-1278.
14. WINDSOR, I. M., and L. M. BLACK. 1973. Evidence that clover club leaf is caused by a rickettsialike organism. Phytopathology 63:1139-1148.