Mycoplasmalike Bodies in Sieve Tubes of Pear Trees Affected with Pear Decline

Hiroyuki Hibino and Henry Schneider

Visiting Assistant Plant Pathologist, and Plant Pathologist, respectively. Department of Plant Pathology, University of California, Riverside 92502.

Research accomplished while the first author was on leave from the Institute for Plant Virus Research, Chiba, Japan.

Accepted for publication 20 October 1969.

ABSTRACT

Leaf veins of pear decline-affected trees (*Pyrus* sp. 'Variolosa') were examined with the electron microscope. Mycoplasmalike bodies were found in the sieve tubes of swollen brown veins of old leaves, but were not abundant in newly formed leaves before the brown discoloration appeared. Absence of mycoplasmalike bodies in healthy pear trees suggested a possible role of these bodies in the etiology of pear decline. Phytopathology 60: 499-501.

Pear decline is a destructive disease of *Pyrus*, but the causal agent remains unknown. The pathogenic entity is transmitted by grafting (1, 8, 9) and by *Psylla pyricola* (4). The transmissible factor has been postulated to be a virus, but particles resembling viruses have not been found in diseased pear tissue. In the course of anatomical and cytological investigations, we found mycoplasmalike bodies in the sieve tubes of pear decline-affected trees similar to those in some "yellows type" diseased plants (2, 5).

Pear decline was initially described as a bud-union disease affecting certain scion rootstock combinations (6). In the greenhouse, the major lateral veins of old leaves of *Pyrus* sp. 'Variolosa' become swollen and brown (8, 10, 11). It was thought that these swollen veins would be more useful diseased structures in which to find the causal agent than the pathological tissues at bud-unions. The latter may be a secondary injury not directly caused by the causal agent.

MATERIALS AND METHODS.—In the current investigations, Variolosa pear trees grown from cuttings were inoculated using pear psylla in July 1966 by George Kaloostian, Entomology Research Division, ARS, USDA. In 1967 and 1968, the inoculated trees showed brown leaf vein symptoms. After being held at 3 C in the winter of 1968, they were moved to the greenhouse on 3 January 1969. From the new shoots, five leaf samplings for electron microscopy were made at irregular intervals between 20 February and 5 June 1969. From diseased and healthy leaves, a major lateral vein and the adjacent mesophyll were excised, fixed in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 4 hr, and postfixed in buffered 1% osmium tetroxide for 3 hr or overnight. After dehydration in an alcohol series, they were embedded in Epon and sectioned with a diamond knife mounted on a Porter Blum MT-1 or MT-2 microtome. The sections were stained with uranil acetate and lead citrate, and examined with an Hitachi HU-11A electron microscope.

RESULTS AND DISCUSSION.—The pathology of brown leaf veins of Variolosa is characterized by necrosis of the sieve tubes, excessive phloem formation, and accumulation of starch in the leaf cells (11). Although we searched for viruslike particles in the leaf veins of the *Psylla*-inoculated Variolosa, we found none. We first became aware of mycoplasmalike bodies in the sieve tubes of tissues beginning to show brown veins. Re-examination of earlier prepared sections from the collections made at intervals revealed the following relationship between the occurrence of mycoplasmalike bodies and the symptoms of brown vein. We found no bodies in the lateral veins of newly formed leaves collected on 20 February; they were sparsely present in the leaf-vein sieve-tube elements of newly matured leaves collected on 13 and 21 March 1969. Symptoms of brown vein first appeared about 5 April on older mature leaves. Collections of these on 1 May and 5 June 1969 revealed that many of the sieve tubes were necrotic, and collapsed. In some of the sieve tubes that remained functional, mycoplasmalike bodies were abundantly present. The bodies were limited to mature sieve tubes, with none being observed in young sieve-tube elements, companion cells, or parenchyma cells. Mycoplasmalike bodies were not found in control trees.

The structure of these bodies (Fig. 1, 2) was similar to mycoplasmalike bodies described in various plants affected with yellows-type disease (2, 3, 5, 7). The mycoplasmalike bodies were bound by a poorly defined membrane with some amorphous material attached externally. There were two types of bodies. Those of one type were spherical to oblong, 50-800 nm in diam, and the central area was fairly electron-transparent, with strands traversing it. Ribosomelike particles, 10-15 nm in diam, were located peripherally. The other type were spherical, 50-400 nm in diam, and contained an electron-dense ground substance with ribosomelike particles distributed uniformly throughout the cell. Elongated bodies like those observed in other mycoplasma-affected plants (5) were not observed. Some of the bodies appeared to be splitting by binary fission.

Mycoplasmalike bodies were not found in healthy trees, and were not abundant in diseased trees until the brown discoloration of veins appeared. These facts suggest a possible role of these bodies in the etiology of pear decline. If they are the causal agent, this will be the first known instance of a psyllid-borne mycoplasma.

Mycoplasmalike bodies were also found in small brown veinlets of *Pyrus communis* L. 'Anjou', in major lateral veins of a seedling of *Pyrus serotina* Rehd.

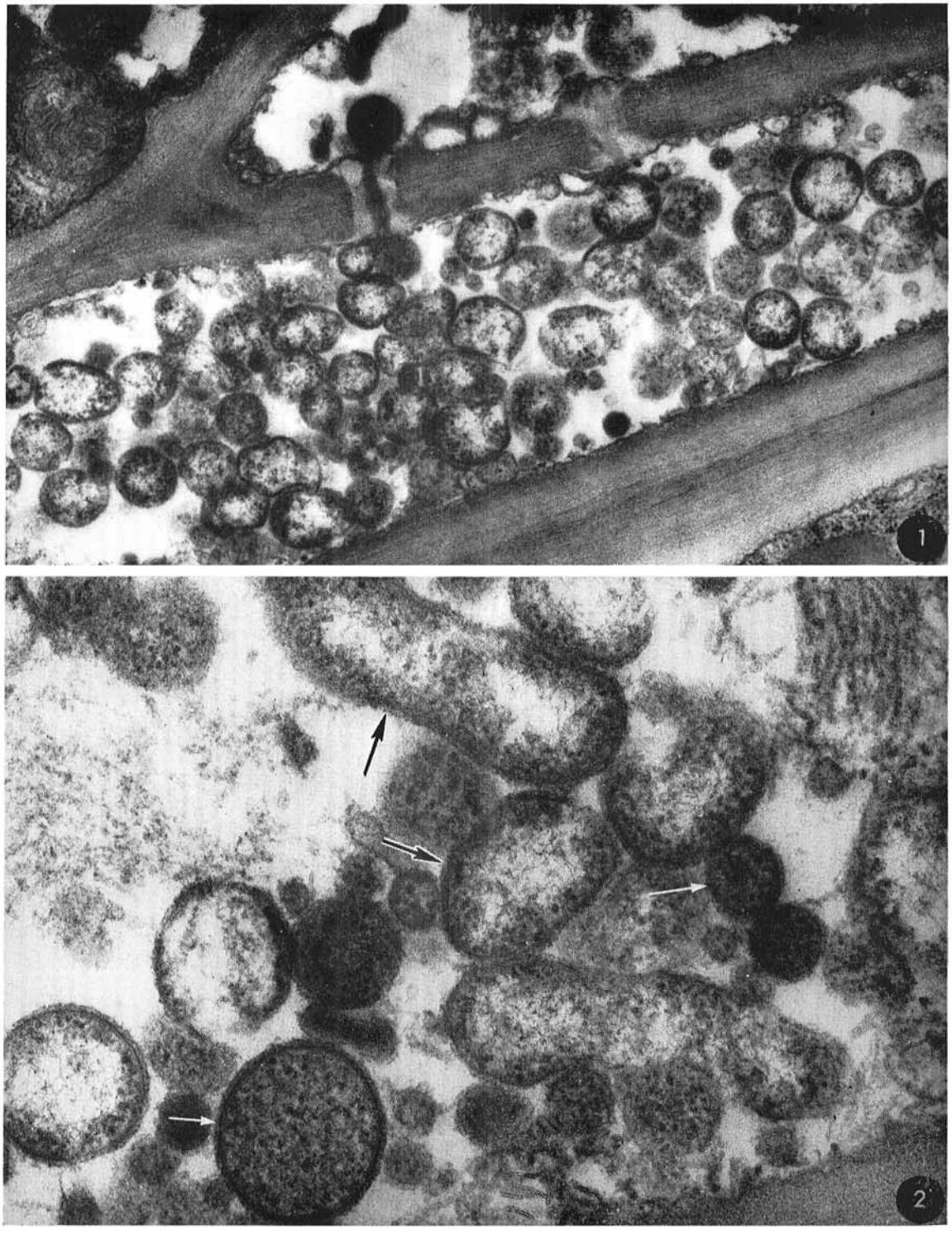

Fig. 1-2. Cross sections of sieve-tube elements of brown leaf veins of Variolosa affected by pear decline. The elements contain mycoplasmalike bodies of two types. **1)** Contiguous sieve-tube elements showing distribution of mycoplasma (×29,000). **2)** Mycoplasmalike bodies with two types of inner structures. One type with electron-translucent center with strands; ribosomelike particles are mostly at the periphery (black arrows). The other type of bodies are filled with electron opaque substances and ribosomelike particles which are randomly distributed (white arrows) (×75,000).

'Chojuro', and in Angers Quince A, all affected by pear decline. The trees were previously described (8).

LITERATURE CITED

1. BLODGETT, E. C., M. D. AICHELE, & J. L. PARSONS. 1963. Evidence of a transmissible factor in pear decline. Plant Dis. Reptr. 47:89-93.
2. DOI, Y., M. TERANAKA, K. YORA, & H. ASUYAMA. 1967. Mycoplasma- or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. Ann. Phytopathol. Soc. Japan 33:259-266.
3. GRANADOS, R. R., K. MARAMOROSCH, & E. SHIKATA. 1968. Mycoplasma: Suspected etiologic agent of corn stunt. U.S. Nat. Acad. Sci. Proc. 60:841-844.
4. JENSEN, D. D., W. H. GRIGGS, C. Q. GONZALES, & H. Schneider. 1964. Pear decline virus transmission by pear psylla. Phytopathology 54:1346-1351.
5. MARAMOROSCH, K., E. SHIKATA, & R. R. GRANADOS. 1968. Structures resembling mycoplasma in diseased plants and in insect vectors. Trans. New York Acad. Sci., Series II. 30(6):841-855.
6. NICHOLS, C. W., H. SCHNEIDER, H. J. O'REILLY, T. A. SHALLA, & W. H. GRIGGS. 1960. Pear decline in California. Calif. Dep. Agr. Bull. 49:186-192.
7. PLOAIE, P., & K. MARAMOROSCH. 1969. Electron microscopic demonstration of particles resembling mycoplasma or Psittacosis-Lymphogranuloma-Trachoma group in plants infected with European yellow-type diseases. Phytopathology 59:536-544.
8. SCHNEIDER, H. 1970. Graft transmission and host range of the pear decline causal agent. Phytopathology (in press).
9. SHALLA, T. A., L. CHIARAPPA, & T. W. CARROLL. 1963. A graft-transmissible factor associated with pear decline. Phytopathology 53:366-367.
10. TSAO, P. W., H. SCHNEIDER, & G. H. KALOOSTIAN. 1966. A brown leaf-vein symptom associated with greenhouse-grown pear plants infected with pear decline virus. Plant Dis. Reptr. 50:270-274.
11. TSAO, P. W., & H. SCHNEIDER. 1967. Pathological anatomy of pear tissues sensitive to pear decline virus. Phytopathology 57:103.