Witches'-Broom of Willow: Salix Yellows

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ABSTRACT

A witches'-broom disease of *Salix rigida* in New Hampshire and nearby states spreads in nature. Severely diseased branches commonly die in winter. The disease agent has been transmitted to *Salix caprea* by grafting, but natural infection of this species has not been observed. Electron micrographs revealed the presence of typical mycoplasmalike bodies in phloem elements of diseased, but not of healthy, plants.

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Additional key words: mycoplasma, electron microscopy.

RESUMEN

Un "mal de escoba de brujas" capaz de propagarse bajo condiciones naturales suele afectar el sauce, *Salix rigida*, en Nueva Hampshire y estados vecinos. Las ramas muy afectadas generalmente mueren en el invierno. El agente causante fue transmitido a *Salix caprea* mediante injertos a pesar de que esta especie mostraba resistencia en su estado natural. Una serie de electronografías revelaron la presencia de micoplasma en el floema de plantas afectadas pero no en el de plantas sanas.

A new witches'-broom type of disease has been observed occasionally in the wand willow, *Salix rigida* Muhlenberg, in widely scattered locations throughout southern New Hampshire. The disease has also been observed in New York and Massachusetts. Usually no more than one affected plant is found in each location, although healthy plants of the same species are often numerous in the vicinity. The disease is characterized by the breaking of dormancy of axillary buds and consequent growth of numerous spindly, erect branches bearing leaves that are, in general, smaller than those on healthy plants.

The witches'-brooms formed in *S. rigida* by the development of numerous axillary buds often die in winter, presumably as a result of lack of adequate dormancy in the affected twigs. The less-affected basal parts of the diseased plants survive year after year, and develop some affected branches as well as some normal-appearing branches each year.

A single affected plant of *S. rigida* was transplanted to an area where healthy plants of this species were being used to prevent soil erosion by wave action at the edge of a lake. A year later, several nearby plants became diseased, and within a few years, plants located some 15 or 20 feet away were exhibiting witches'-brooms.

Scions taken from affected plants of *S. rigida* were grafted onto recently rooted cuttings of the European pussy willow, *S. caprea* L. Systemic infection of the understocks occurred eventually in almost all cases. The induced disease was characterized by initial clearing of veins and subsequent breaking of dormancy of many axillary buds with formation of witches'-brooms consisting of spindly erect branches. Often the first symptoms of graft-transmitted disease appeared within 2 weeks, but in some cases, evidence of transmission did not appear until much later. In one case, the disease remained confined to the introduced scion. Despite the proven susceptibility of *S. caprea* to infection by scion grafts, no instance of natural spread to this species has been observed.

The partial localization of disease in affected plants has made it possible for workers to derive healthy clones of *S. rigida* from diseased plants by selecting twigs that appeared normal, making cuttings from them, rooting these cuttings, and discarding any that subsequently show symptoms. It is clear that the causal agent is actually absent from some parts of the affected plants, even in plants showing conspicuous disease symptoms for several years.

The symptoms of vein clearing in newly infected plants, the breaking of dormancy of axillary buds, and the growth of erect and spindly axillary shoots suggest that this spontaneously spreading and graft-transmissible brooming disease of willow is a member of the yellows-disease group and comparable to aster yellows, peach yellows, locust witches'-broom, and cranberry false blossom in its etiology. No insect vector of Salix yellows disease has been identified. The yellows diseases, as a group, have been characteristically transmitted by leafhoppers of the family Cicadellidae, and it is possible that one or more species of this family is present and capable of transmitting the observed yellows disease of *S. rigida* in nature.

The detection of mycoplasmalike bodies in some 40 plant diseases of the yellows or witches'-broom type (3) prompted a search for similar bodies in diseased portions of *S. rigida*. In September 1970, midveins of leaves from diseased trees, collected in

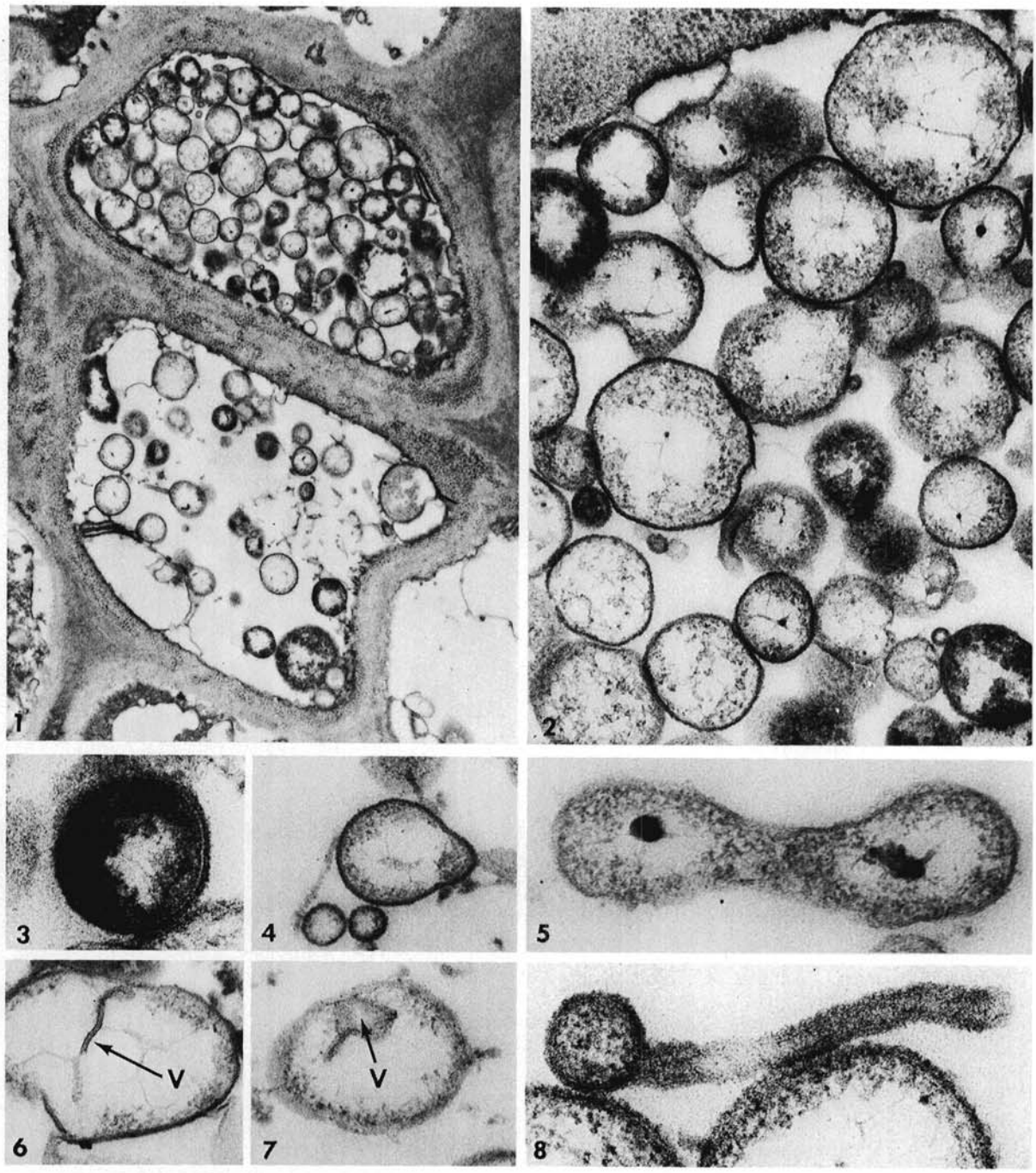

Fig. 1-8. Mycoplasmalike bodies in sieve tube elements of midveins from witches'-broom *Salix rigida* leaves. **1)** Two adjacent sieve tube elements. The upper contains a high accumulation of pleomorphic bodies; and the lower, scattered bodies (× 12,400). **2)** Portion of the sieve element containing mycoplasmalike bodies of varying electron density and size (× 48,000). **3)** A small spherical body containing cytoplasm of high electron opacity (× 105,000). **4)** A small body with scattered cytoplasm and nuclear strands (× 54,300). **5)** A mycoplasmalike body apparently undergoing binary fision. Note the nuclear strands and the dense center, as well as ribosomes at the periphery (× 70,000). **6,7)** Mycoplasmalike bodies containing vacuoles (V). Arrows indicate layers of high electron density surrounding the well-delineated unit membranes of the bodies. Note the closed (6) and open (7) vacuoles (× 54,300). **8)** A filamentous body extruding from a small spherical body (× 105,000).

southern New Hampshire, were prepared for electron-microscopic observation according to described procedures (2), except for two modifications: primary fixation in 3% glutaraldehyde lasted for 4 hr, followed by 18 hr of rinsing in the buffered solution.

Pleomorphic bodies resembling mycoplasmas were detected in sieve tube elements of diseased leaves (Fig. 1). No mycoplasmalike bodies were present in neighboring phloem cells. At a high magnification, the typical morphology of mycoplasmas was confirmed. The diversity in electron opacity as well as in shape and size is illustrated in Fig. 1-4. The nuclear area, lacking a nuclear envelope, was comprised of deoxyribonucleic acidlike strands varying in electron permeability (Fig. 2, 4, 5, 6). The mycoplasmalike microorganisms were bounded by unit membranes, triple-layered, and comprised of two electron-opaque layers with an electron-lucent layer between (Fig. 2, 5, 8). The bounding unit membrane was ca. 10 nm thick. An electron-opaque layer surrounded the unit membrane of the bodies (Fig. 2, 3, 4, 6, 8). This type of layer has also been illustrated by others (1). The layer may be characteristic for certain species of mycoplasmalike microorganisms that infect plants, or it may be formed by woody plant hosts, the only ones in which this feature has been noted. Ribosomes of the presumptive mycoplasmas were smaller than those of the host cells. Some bodies contained so-called vacuoles (Fig. 5-7). Filamentous bodies extruding from spherical bodies of various sizes often were encountered (Fig. 8). Mycoplasmalike bodies, apparently undergoing binary fision, were also observed (Fig. 5).

No mycoplasmalike bodies were found in the phloem, xylem, or parenchyma of healthy *Salix*, nor were structures resembling viruses or bacteria observed in healthy or diseased plants. The finding of mycoplasmalike bodies in witches'-broom of *Salix* suggests that these bodies may be the causative agents of the disease. Therefore, the newly described disease is classified as "Salix yellows".

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