Mycoplasmalike Bodies Found in Phloem of Black Locust Affected with Witches'-Broom

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ABSTRACT

Pleomorphic bodies resembling mycoplasmas were found in the phloem tissues of black locust trees (*Robinia pseudoacacia*) affected with witches'-broom. These bodies ranged in size from 90 to 1,000 nm. They contained ribosomelike bodies and strands typical of mycoplasma DNA. They were delimited by a trilaminar membrane. No mycoplasmalike bodies were observed in the phloem tissues of healthy black locust.

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Additional key words: tree virus, yellows disease.

Black locust witches'-broom was one of the earliest recorded viruslike disorders of forest trees. First described in 1898 on locust stump sprouts in Maryland (12), the disease has since been found

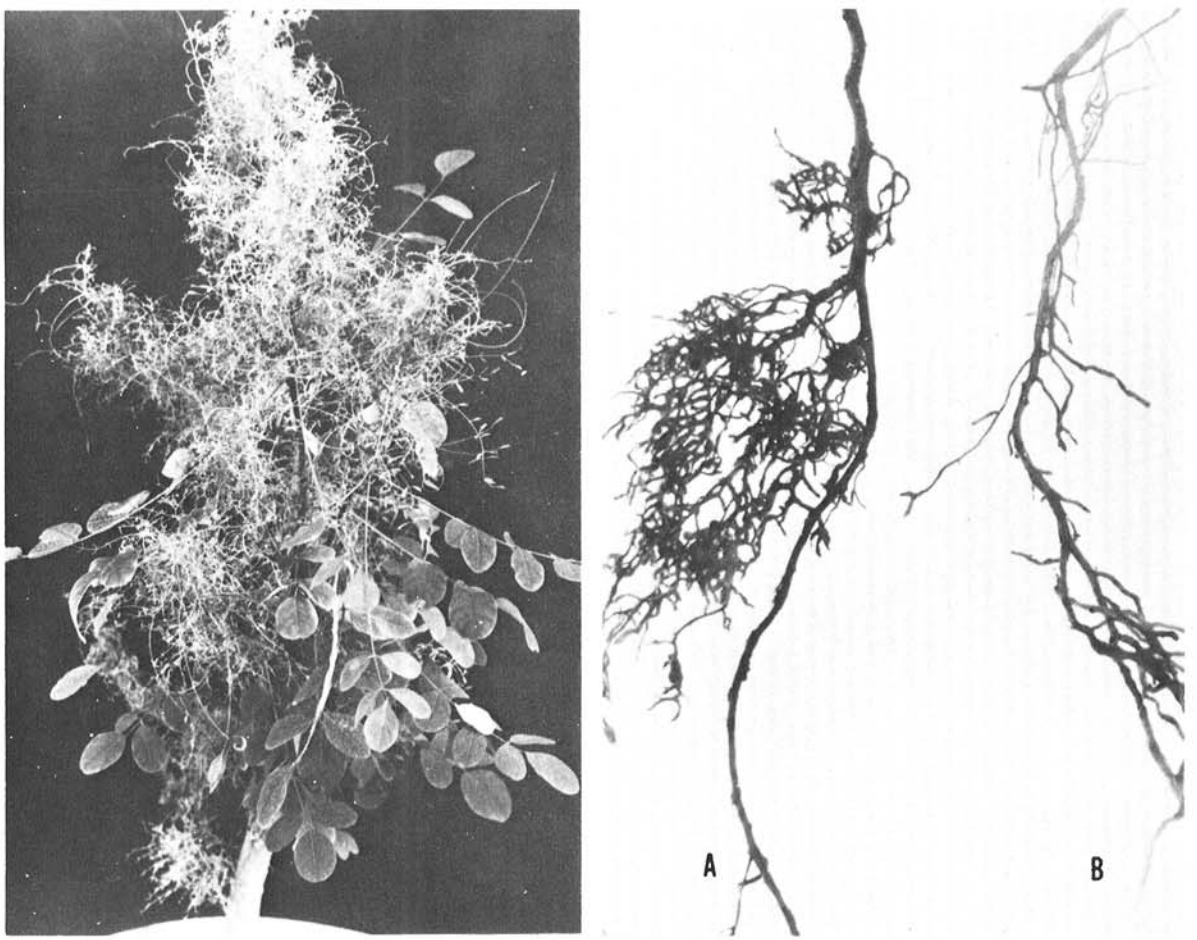


Fig. 1. (Left) Severely broomed root sprout of black locust. As axillary buds are forced into growth, many small succulent shoots bearing leaves with tiny leaflets appear on curved petioles. Old leaves drop soon after the axillary buds develop. (Right) Brooms on diseased root (A) show extensive rebranching of second-order roots. Healthy root (B) shows normal branching.

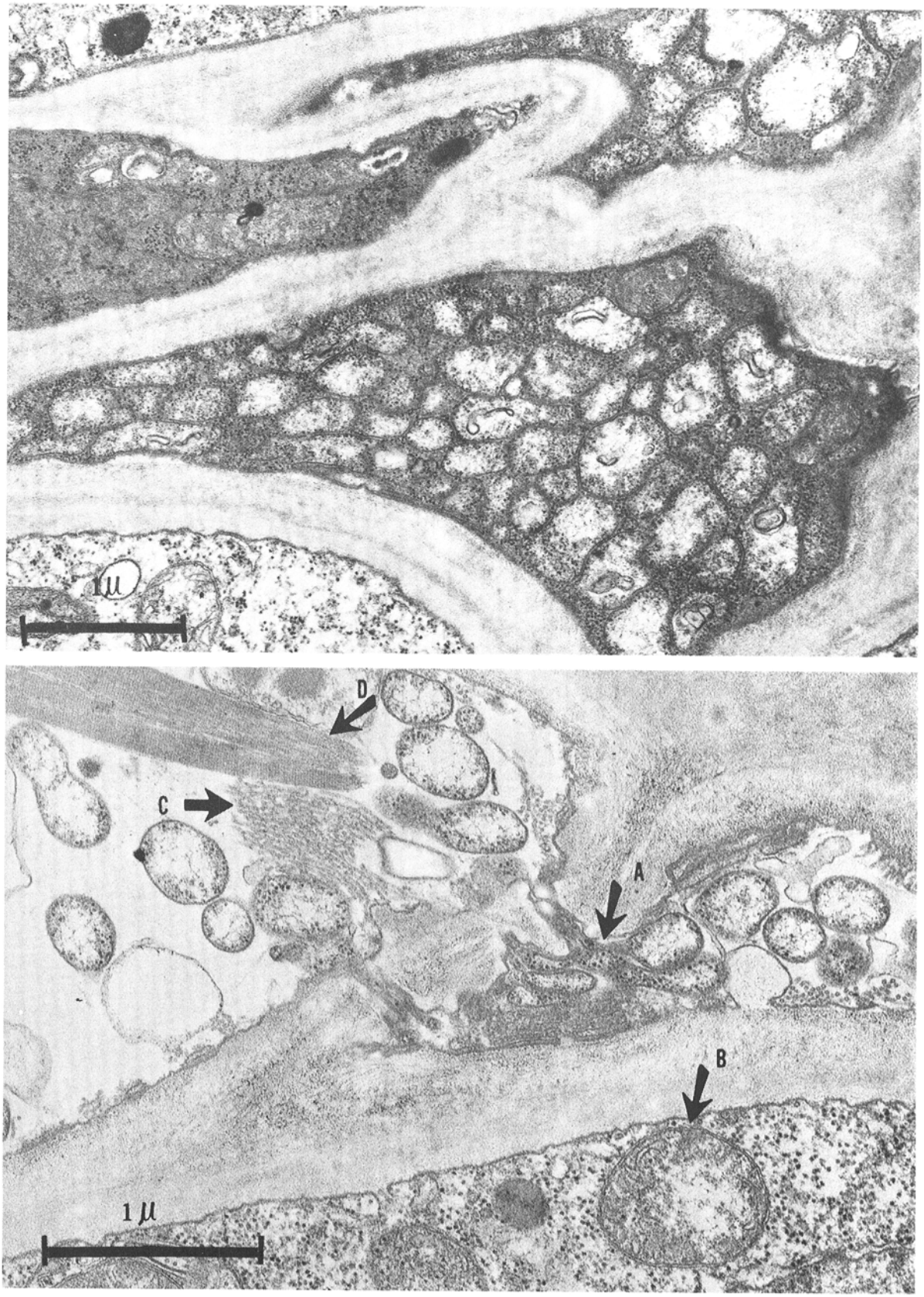
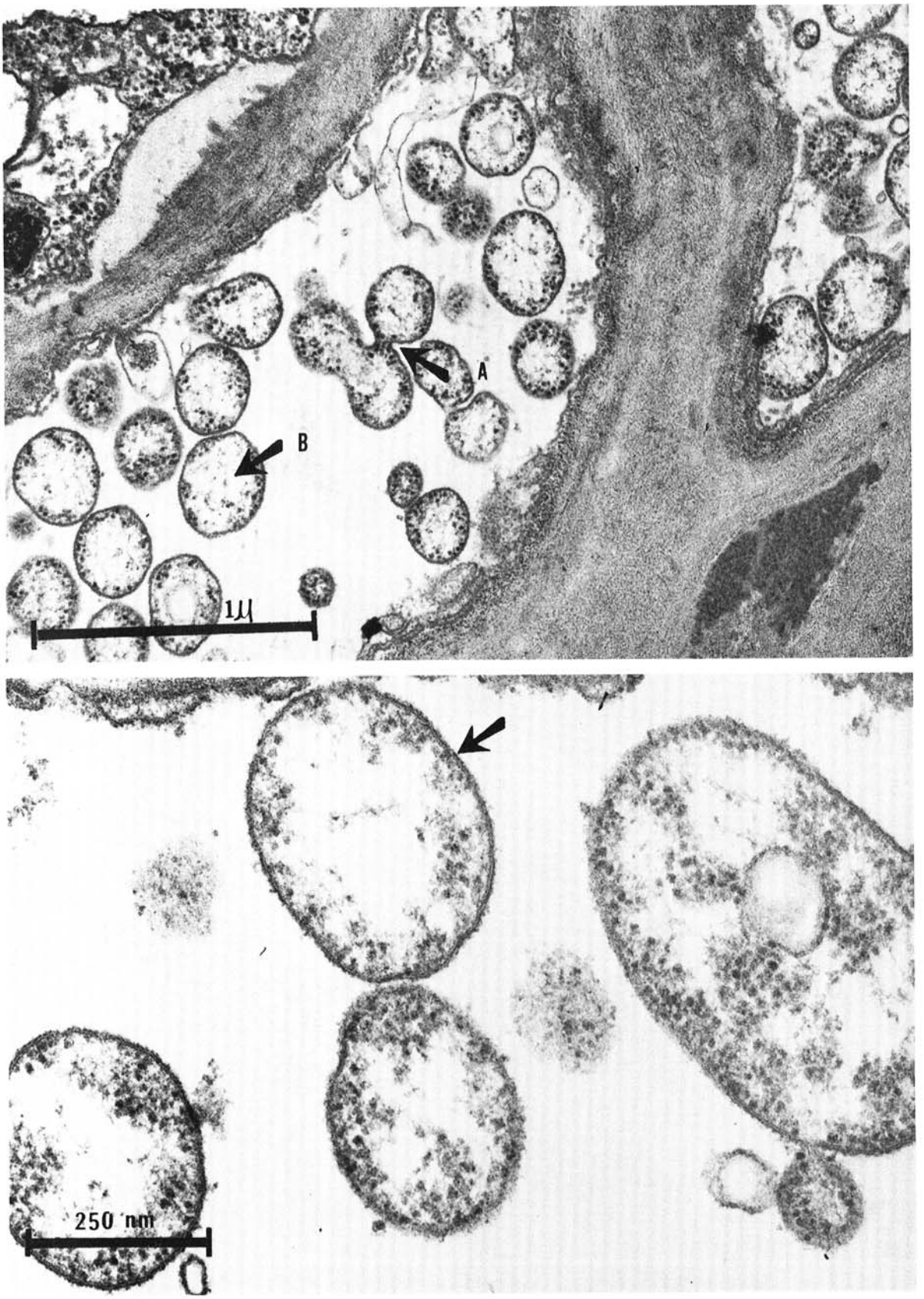


Fig. 2. (Above) Some phloem elements of black locust affected with witches'-broom are packed with mycoplasmalike bodies. (Below) Mycoplasmalike bodies (A) passing through sieve plate are seen as narrow tubular bodies; mitochondrion (B); P-protein (C); and unidentified artifact (D).


A
B
1μ
250 nm

throughout the eastern USA as well as in several European countries (10). This yellows-type disease was presumed to be caused by a virus when transmission was demonstrated by grafting (2, 8). No vector is known.

Characteristically, black locust witches'-broom results in systemic brooming of root and stump sprouts, roots, and less frequently the crowns of affected trees (Fig. 1). Broomed shoots are usually erect, and their leaves are much reduced in size. Vein-clearing is characteristic of affected leaves, but chlorosis does not occur.

Brooms usually result from the abnormal proliferation of normally dormant axillary buds in leaf axils of affected shoots or branches. Because brooms commonly do not form until late summer, they continue growth into late fall and frequently die back during the winter.

An infected tree may send up severely broomed root sprouts each year, yet show no crown symptoms. A tree producing broomed shoots one year may produce normal-looking shoots the following year. Eventually, severely broomed branches die back from the tips.

Since the work of Doi et al. (1), the supposed virus etiology of yellows diseases of plants has been in question. To date, mycoplasmalike bodies have been found associated with more than 40 yellows disorders in plants (6). Among these are several important diseases of forest trees (1, 4, 5, 7, 11, 13).

This paper is a report on the results of our cytological investigation of black locust, *Robinia pseudoacacia* L., affected with witches'-broom.

MATERIALS AND METHODS.–Diseased black locust branches, roots, and root sprouts showing varying degrees of brooming were collected in a woodlot near Front Royal, Va., in 1970 and 1971. Healthy material was collected from seedling trees grown in our experimental nursery at Delaware, Ohio.

Petiole tissue and inner bark from healthy and diseased material were cut into small bits and fixed for 12 to 18 hr at 4 C in 3% 0.1 M, pH 6.8, phosphate-buffered glutaraldehyde. The tissues were then washed in buffer and postfixed in 2% buffered osmium tetroxide for 2 hr at ca. 70 F. After another wash, the tissues were dehydrated in a graded ethanol series and placed in propylene oxide. Reinfiltration was done by the use of graduated concentrations of Epon 812 in propylene oxide. Finally, the tissues were immersed in 100% Epon, placed in Beem capsules or flat molds, and cured for 24 hr at 35 C, 48 hr at 45 C, and 72 hr at 60 C.

Thin sections of embedded tissues were cut with an LKB Ultratome III, using a diamond knife, and stained in a 5% aqueous solution of uranyl acetate for 0.5 to 1 hr, followed by 1 to 5 min in Reynold's lead citrate (9). Examinations were made with an Hitachi HU-11E electron microscope.

RESULTS AND CONCLUSIONS.–Mycoplasma-like bodies (MLB) were observed in sieve elements and companion cells in phloem of stems, roots, and petioles of black locust exhibiting witches'-broom symptoms. In some cells, MLB were very numerous, occupying most of the lumen (Fig. 2, above), but in others they were completely lacking. MLB were most abundant in phloem tissues of brooms expressing the most advanced symptoms of decline; yet in some tissues obtained from slightly broomed shoots, they were difficult to find.

The MLB were most commonly oval or nearly spherical in shape, although filamentous forms were occasionally seen, particularly near the sieve plates. In some cases, the filamentous forms seem to accomplish cell-to-cell passage through the narrow slits in the sieve plate (Fig. 2, below). Small, more densely staining bodies about 80 nm in diam were also seen. As others have reported (6), some bodies appeared to be dividing by fission (Fig. 3, above). Although varying greatly in size (90 to 1,000 nm in the largest dimension), the more typical oval forms averaged ca. 200 to 350 nm in length.

As is characteristic of most mycoplasmas, the MLB found in black locust have a unit membrane approximately 8 to 10 nm thick (Fig. 3, below) and contain peripheral ribosomelike bodies and centrally located threadlike material resembling the DNA of animal mycoplasmas (3).

The association of MLB with black locust witches'-broom lends support to the hypothesis that these mycoplasmas play a role in the etiology of yellows-type diseases. Though still circumstantial, the rapidly growing number of such associations reported in the literature in a wide range of plants strengthens this supposition.

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Fig. 3. (Above) Typical mycoplasmalike bodies in sieve elements of broomed black locust. Some bodies (A) appear to be dividing by fission. Strands of fibrillar material (B), probably DNA, appear in most bodies. (Below) Trilaminar unit membrane (arrow) of mycoplasmalike bodies in diseased locust is evident in this electron micrograph.

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