Mycoplasmalike Organism Associated with Pecan Bunch Disease

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Accepted for publication 8 March 1974.

ABSTRACT

Pecan bunch is a widespread and serious disorder occurring throughout the commercial range of southern pecan, *Carya illinoensis*. It was formerly presumed to be virus induced, but the consistent association of a mycoplasmalike organism (MLO) with diseased and not with healthy trees strongly points to MLO etiology. Unlike some other mycoplasma diseases, pecan bunch is not systemic throughout the tree. The MLO are found in phloem sieve elements and companion cells of stems and leaves of affected trees. They are most abundant in severely broomed material and are rarely observed in nonsymptomatic tissues. They are pleomorphic, vary in size from 80 to 800 nm, contain ribosomal bodies and fibrillar material typical of DNA, and are delimited by a tripartite unit membrane. Spherical and oval forms are most common but filamentous forms can be seen near sieve plates.

Phytopathology 64:1269-1272.

Additional key words: yellows disease, tree virus, ultrastructure.

Pecan bunch, a yellows-type witches' broom disease, was first reported in 1937 near Shreveport, Louisiana (3). Now known in eight southern states, bunch disease is a serious and widespread disorder of native pecan, *Carya illinoensis* (Wang.) K. Koch., and a number of its horticultural cultivars. The same or a similar disease also occurs in Louisiana on other native hickory species, including *C. aquatica* (Michx. f.) Nutt., *C. ovata* (Mill.) K. Koch., *C. cordiformis* (Wang.) K. Koch., and *C. tomentosa* Nutt. In several instances, bunch-diseased pecan trees in an orchard appear to result from spread of the disease from one or more of these hickory species (3).

Pecan rosette, caused by zinc deficiency, and pecan bunch were not recognized as separate disorders before 1932 (3). Both cause brooming on terminal shoots, but, in addition, tufts of willowy shoots arise directly from main limbs and branches of bunch-diseased trees. However, the distinct difference between the two was not recognized until pecan rosette was corrected by treatment with zinc sulfate (1,4) and graft transmission experiments (3) demonstrated that only the bunch disease was infectious. On the basis of symptoms, transmission by grafting, and failure to incriminate other organisms, bunch disease was assumed to be caused by a virus.

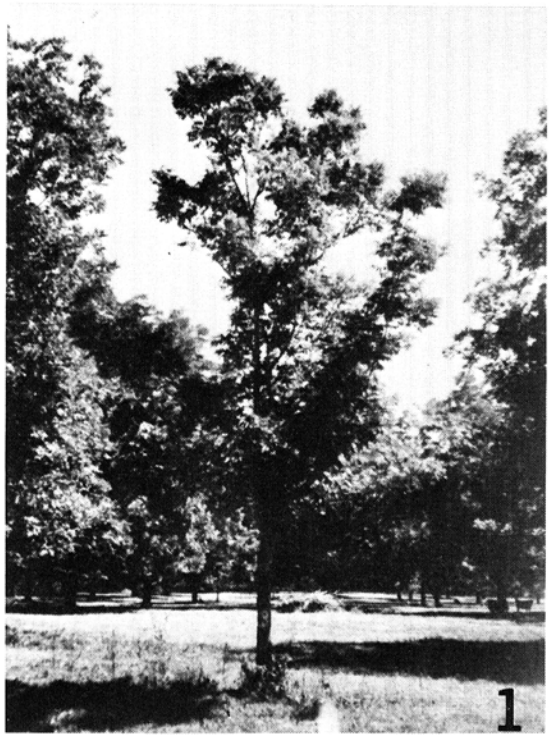
As with native seedling pecans, commercial pecan cultivars vary widely in susceptibility. Characteristically, however, from one to many brooms, each consisting of several or more slender shoots, appear on diseased trunks and branches of susceptible cultivars (Fig. 1). These shoots tend to stand more nearly erect than normal (3, 9). They come into leaf before normal foliage in the spring and tend to continue growth late in the fall (Fig. 2). As a result they are often damaged or killed by winter cold. Brooms apparently arise from the precocious and indeterminate development of dormant adventitious or axillary buds. Often this witches' broom disease affects one or more branches on a tree without damaging the yield or quality of nuts on healthy parts. Leaves on bunched twigs are often broader than normal and sometimes distorted and chlorotic, but this varies with the variety. Sometimes some of the catkins are broomed.

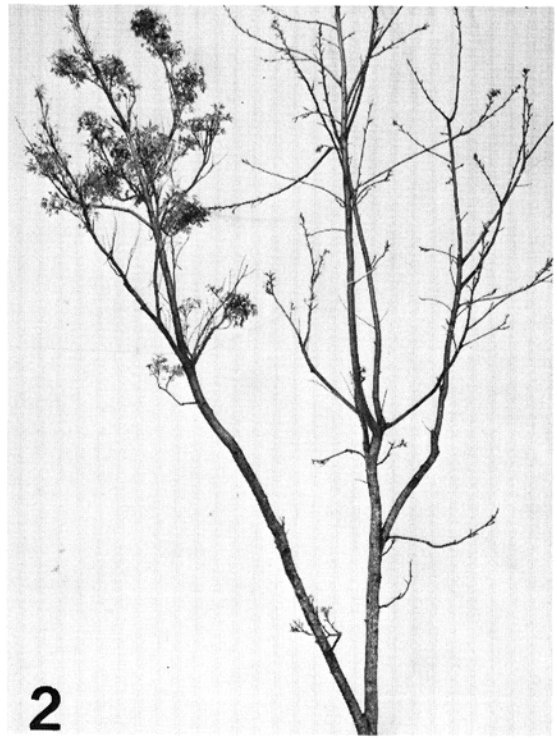
The relatively recent discovery of mycoplasmalike organisms in plants affected with yellows diseases (5), has stimulated considerable interest in this little-known group of probable plant pathogens. Since their taxonomic positions have not yet been clarified, this group, along with certain rickettsia- and spirochaetelike organisms have recently been referred to as yellows disease-associated agents (YDAA) (6). Reported in this paper are the results of our electron microscopic investigations showing the association of mycoplasmalike organisms (MLO) with southern pecan infected with bunch disease.

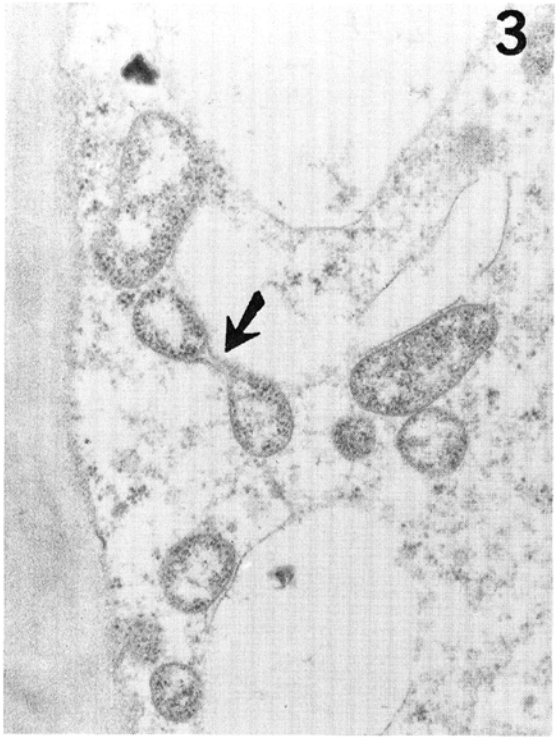
MATERIALS AND METHODS.—Beginning in 1970, specimen material from pecans was collected periodically from several different orchards in Louisiana. This material consisted of leaves and twigs cut from healthy trees and from the brooms of infected trees. Most material examined was from native pecan or from the pecan cultivar Mahan, a cultivar highly susceptible to bunch disease.

Small pieces of tissue, about 1 mm square, were cut from the inner phloem, rachis, and petioles of diseased and healthy twigs. These were fixed in 3% 0.1 M, pH 6.8, or pH 7.2 sodium phosphate-buffered gluteraldehyde for 2 h at room temp. The tissues were then washed several times in sodium phosphate buffer and postfixed in 2% buffered osmium tetroxide for 2 h at ca. 20 C. Following further buffer washes, the tissues were dehydrated in a graded ethanol series and placed in propylene oxide. Infiltration with Epon 812 was accomplished by

PHYTOPATHOLOGY for September (64:1159-1268) was issued 12 September 1974.







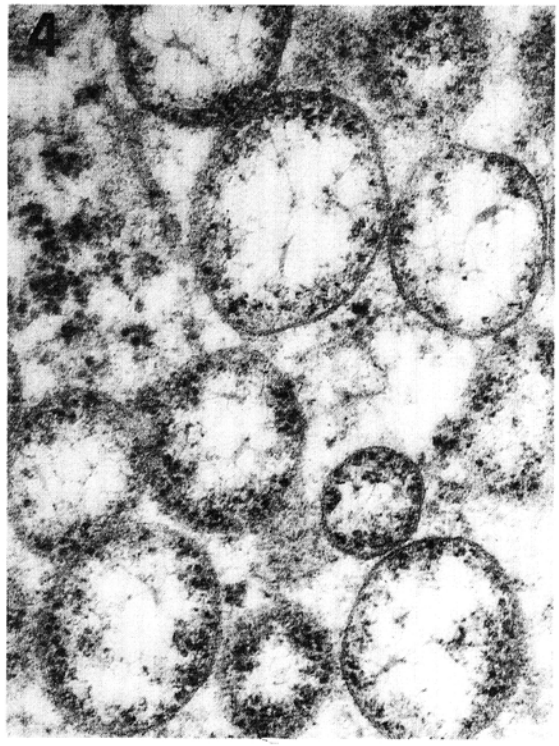
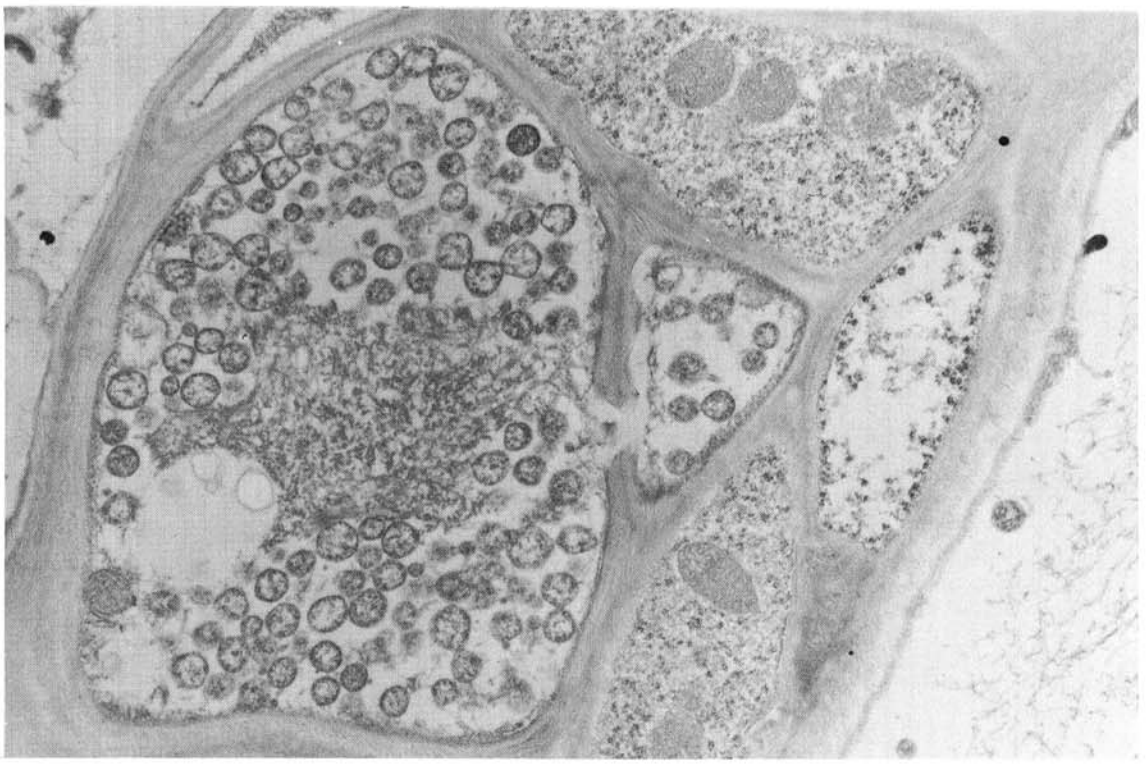


Fig. 1-4. Pecan bunch disease. **1)** Bunched pecan: characterized by the appearance of one to many brooms scattered in the crowns. Shown here is Desirable pecan, a highly susceptible cultivar. **2)** Limb of diseased pecan photographed in early spring showing precocious development of brooms. Development of foliage on the right is normal and is similar to that on healthy trees. **3)** Mycoplasmalike organism apparently dividing by binary fission in sieve element of pecan phloem. × 23,400. **4)** MLO have a trilaminar unit membrane containing fibrillar material, probably DNA, and ribosomes. × 48,800.



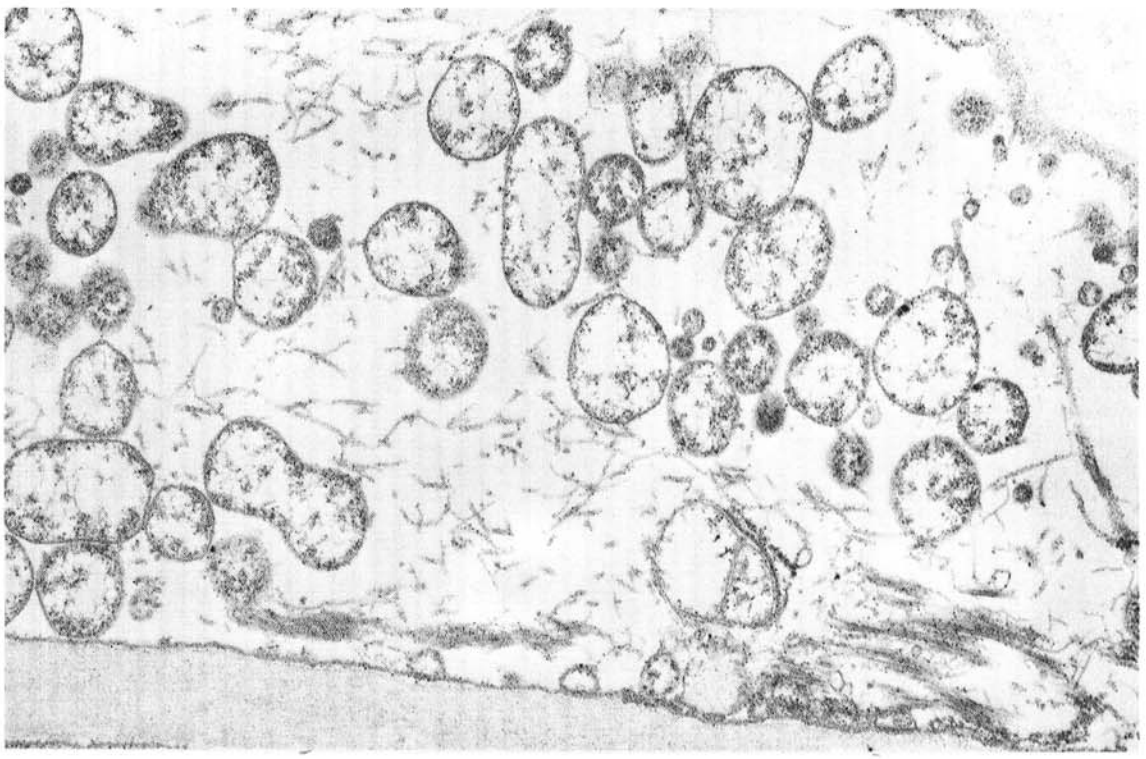


Fig. 5,6. 5) MLO in sieve element and companion cell in petiole of bunch-diseased native pecan. Mitochondria are present in adjacent cells. × 13,430. **6)** MLO in sieve element of pecan phloem. Note septatic cell. Filamentous particles are p-protein. × 26,400.

using graduated concns in propylene oxide. The tissues were embedded in pure Epon 812, placed in Beem capsules, and cured for 24 h each at 30 C, 45 C, and for 72 h at 60 C. Blocks were sectioned with a diamond knife in an LKB Ultratome III, stained 1 h in a 5% aqueous solution of uranyl acetate and for 5 min in Reynold's lead citrate (12). Thin-sections were examined with an Hitachi HU-11E electron microscope.

RESULTS.—Thin-sections of phloem tissue in twigs and petioles of pecan infected with pecan bunch disease revealed MLO in sieve tubes and companion cells (Fig. 5, 6). Their abundance varied widely from cell to cell. As with black locust witches' broom (13), MLO were most abundant in tissues taken from severely broomed twigs. MLO were difficult to find in nonsymptomatic twigs on diseased trees, even though these were located close to brooms, and MLO were not found in healthy trees.

The MLO were usually oval to spherical in shape with occasional filamentous forms in the vicinity of sieve plates. The elongate form is characteristic and perhaps essential in negotiating cell-to-cell movement. Dumbbell-shaped cells suggesting binary fission were often observed (Fig. 3). The average length of the common oval form varied from 150 to 420 nm, while the extremes for all cells measured were 80 to 800 nm. It is questionable that the lower extreme (i.e., 80 nm) represents viable reproductive forms. Most estimates for the minimum size of viable cells range from 125 to 250 nm (2).

The MLO associated with pecan bunch have a unit membrane approximately 10 nm thick, and they contain ribosomes and fibrillar material resembling DNA (Fig. 4). Morphologically, they are similar to other plant MLO reported in the literature.

DISCUSSION.—Like some other yellows-type diseases of trees (7, 8, 11, 13), but unlike most virus diseases, pecan bunch is not generally systemic throughout the tree. This is indicated by the following facts: It spreads slowly within the tree; witches' brooms may occur on one branch or part of the crown for years before symptoms appear on other parts of the tree, and they may never appear; there is evidence to indicate that the disease may be eradicated from mildly diseased pecans by pruning limbs well below the brooms (10); graft transmission is erratic, depending upon the source and nature of the diseased scion material; not only is tissue union required, but transmission also appears to occur only when severely broomed scion material is used for the grafts (3); it does not occur when nonsymptomatic wood from infected trees is used as scion material; and MLO are found consistently and in abundance only in thin-sections from twigs obtained from the witches' brooms; the more conspicuous the brooming, the better the chances of finding MLO.

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