Dodder Transmission of a Mycoplasma from Ash Witches'-Broom

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ABSTRACT

An infectious agent was transmitted from witches'-broom of declining *Fraxinus americana* to *Vinca rosea* by means of dodder. Symptoms in *V. rosea* were typical of yellows-type infection. Transmissibility was confirmed by graftage between infected and healthy *V. rosea,* and by dodder transmission of the agent from yellowed *V. rosea* to *Daucus carota.* Attempts at transmission from sap of yellowed leaves and dodder did not reveal a mechanically-transmissible disease agent. Thin sections of midveins and petioles from yellowed *F. americana, V. rosea,* and *D. carota;* of roots from yellowed *F. americana* and *V. rosea;* and of dodder strands that had been parasitizing yellowed *V. rosea,* were examined by electron microscopy. Typical mycoplasmalike bodies were present, often in large numbers, in the phloem cells of all four species. These bodies were indistinguishable morphologically from mycoplasmas known to cause yellows-type diseases. No virus particles were observed either in the phloem and xylem elements, or in the parenchymatous cells of yellowed leaf tissue. From these results, a mycoplasma etiology is suspected for the witches'-broom disease of ash. Phytopathology 61:151-156.

Additional key words: dieback, yellows disease.

Among declining white ash (*Fraxinus americana* L.) in Dutchess County, N.Y., an occasional tree was noted with symptoms resembling those caused by yellows-type diseases. These included small, chlorotic, sometimes deformed leaves that were often simple rather than pinnately compound (Fig. 1-A,B,C), shortened internodes, abnormally erect branches, and shoot growth from axillary and terminal buds that normally remained dormant until the following year. Branches with this syndrome had a witches'-broom appearance (Fig. 1-A). Although trees in various stages of dieback exhibited this abnormal growth, it occurred predominantly on small branches arising from the trunks of trees in advanced stages of dieback [Class IV (14)]. Two young ash seedlings with many of the above symptoms except dieback were transplanted from the field to the greenhouse in 1962. Despite fertilization, these trees retained their yellows symptoms until their deaths in 1968 and 1970, respectively.

There is abundant evidence that yellows-type diseases are caused by infectious agents transmitted primarily by leafhoppers (Cicadellidae) (16). Electron microscope studies have demonstrated the presence of mycoplasmalike bodies in the phloem elements of a wide variety of herbaceous and woody plants infected with yellows-type diseases (11, 16). Consequently, an effort was made to detect a transmissible agent in ash with yellows symptoms, and to examine diseased tissues for the presence or absence of mycoplasmas.

MATERIALS AND METHODS.—*Demonstration of a transmissible agent.*—Apical cuttings (5-10 cm) of dodder (*Cuscuta subinclusa* Dur. & Hilg.), grown from seed and maintained on healthy tobacco, were attached to witches'-broom formations on five ash trees in the field. The cut ends of the dodder were immersed in vials of water, and the tips were wound around the new shoots. The branches with dodder attached were enclosed in plastic bags fitted with screened ventilation ports to prevent infestation by insects. The dodder formed haustorial connections on all branches, but it remained permanently established on only two of the five trees. Eight weeks after establishment, apical cuttings of dodder from these two trees were transferred to healthy periwinkle (*Vinca rosea* L. 'Little Pinkie') plants in the greenhouse. In addition, another species of dodder (*C. campestris* Yuncker) was established on one of the yellowed ash trees that had been transplanted from the field to the greenhouse. Strands were then trained onto healthy periwinkle, and the dodder connections between the two hosts were maintained for 3 months.

Cuscuta subinclusa that had been growing on healthy tobacco or ash (both grown from seed in the greenhouse) was established on periwinkle for the controls. The dodder shoots were pruned periodically to prevent deterioration of the hosts.

Electron microscopy.—Samples of leaf tissue (1 mm^2) containing both the midvein and interveinal areas, petiole segments (1-2 mm long), and root segments (1-2 mm long) were excised and prepared for thin-sectioning. The specimens were fixed in 3.5% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.3) for 3 hr, washed overnight in fresh buffer, and postfixed for 2 hr in 4% aqueous osmium tetroxide. They were dehydrated for 15 min each in an acetone series of 30, 50, and 70%. After the 70% stage, the samples were transferred to saturated uranyl acetate in 70% acetone and stained for 1 hr. After staining, the specimens were washed in 70% acetone for 3 hr, dehydrated in 90 and 100% acetone, infiltrated overnight in an epon-acetone (1:1) mixture, and embedded in epon. Thin-sections were cut on a Porter-Blum ultramicrotome using a diamond knife, and were examined in a Siemens Elmiskop I at 80 kv without further staining.

RESULTS AND DISCUSSION.—*Demonstration of a transmissible agent.*—Four to 5 months after parasitization

of the periwinkle by dodder, vein clearing and marginal chlorosis developed in leaves of one plant that had supported dodder transferred from one of the field ash with yellows symptoms. These early symptoms were followed by the formation of stunted and chlorotic leaves, the emergence of small flowers with white rather than pink corollas, reduced length of internodes, excessive elongation of axillary shoots, abnormally erect branches, failure of terminal buds to remain dormant with concomitant loss of flower bud initiation, and ultimate wilting and death of the plant (Fig. 1-D).

This transmission experiment was repeated the next year with five additional declining ash trees with yellows symptoms. The same syndrome developed on one periwinkle that had been parasitized by dodder transferred from one of four ashes upon which it had become established. This essentially repeated the results obtained the previous year.

The periwinkle that had been parasitized by *C. campestris* bridged from the yellowed ash in the greenhouse developed typical yellows symptoms 3-4 months after the dodder bridge was severed. The controls did

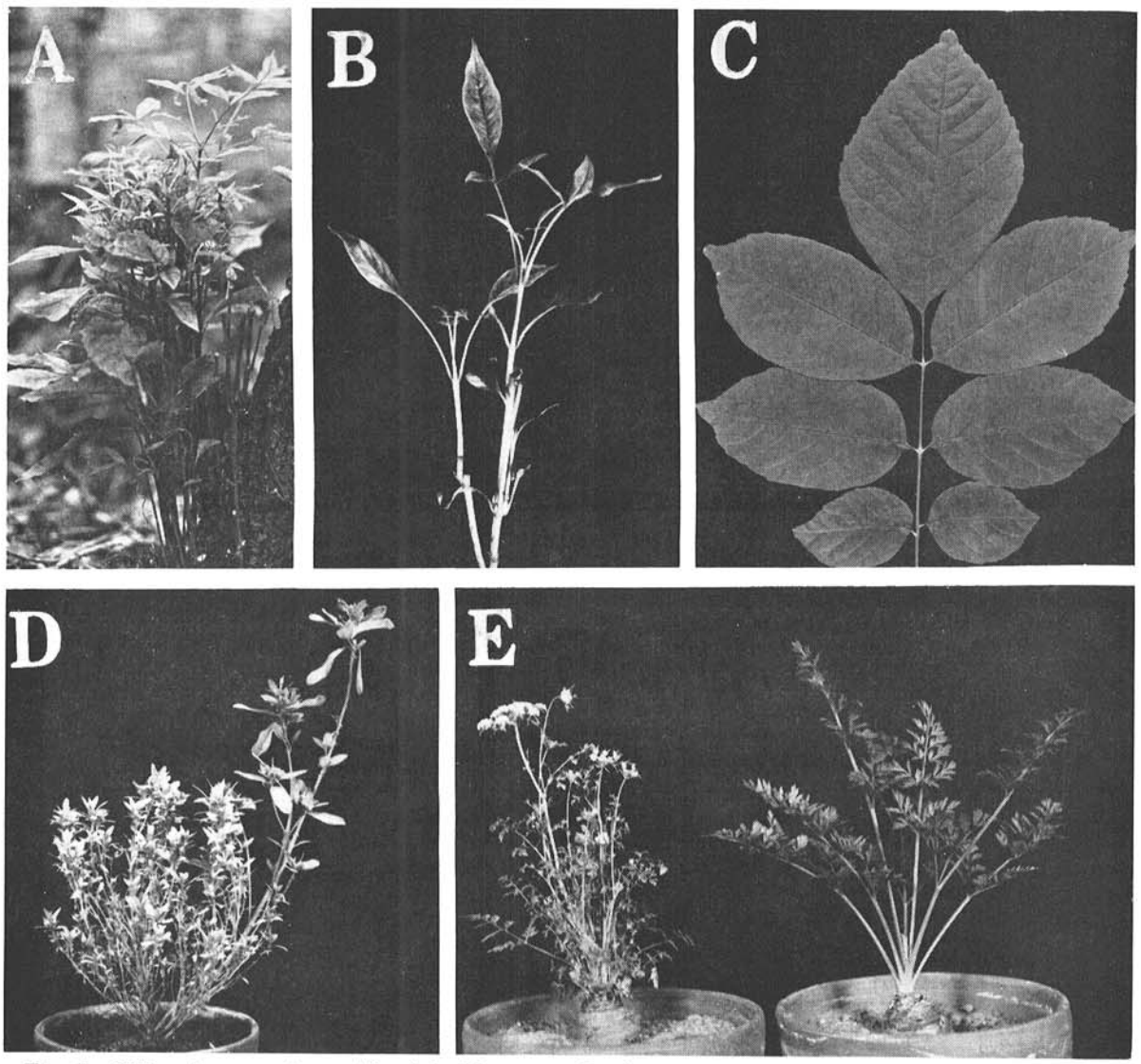
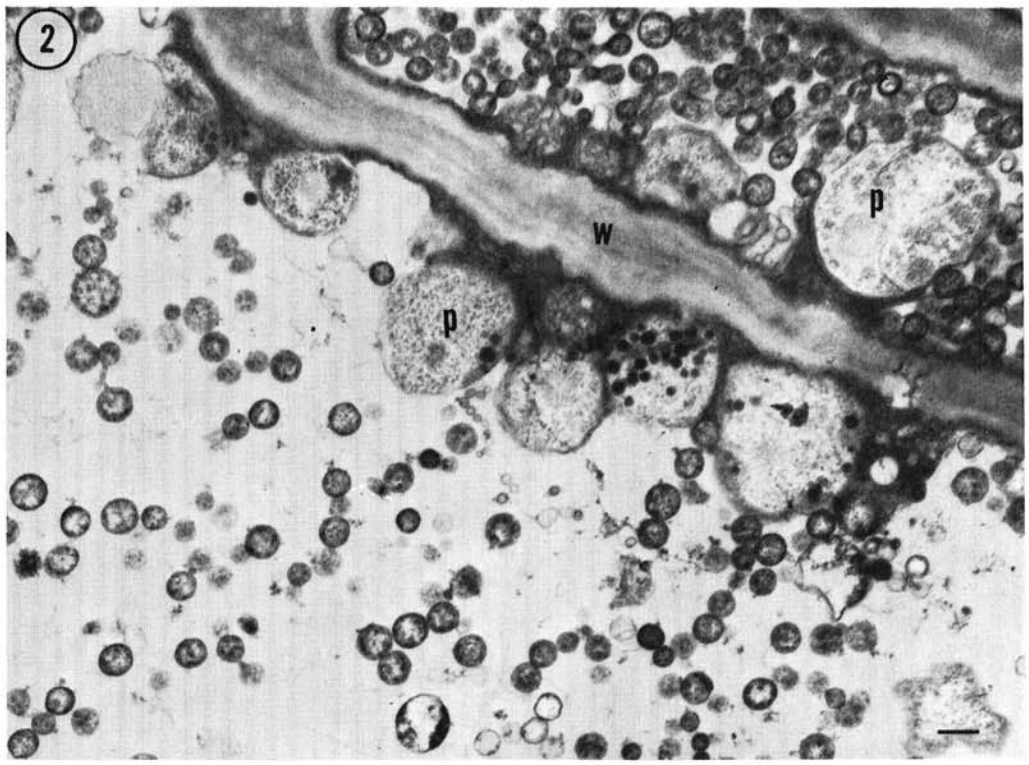


Fig. 1. Yellows-type symptoms: **A)** witches' broom on *Fraxinus americana* trunk (natural infection); **B)** shoots from above ash witches'-broom with abnormal leaves and indeterminate apical growth; **C)** leaf of healthy ash; **D)** *Vinca rosea* inoculated by grafting and showing yellows symptoms except in two branches on right; **E)** *Daucus carota*, infected by dodder transmission (left), and healthy (right).

Fig. 2-3. Mycoplasmalike bodies in phloem sieve tubes of *Fraxinus americana* naturally infected with a yellows-type disease. **2)** Bodies are predominantly spherical. **3)** Elongated and irregular forms. Note the double membrane or two membranes (small arrows) on several of the smaller bodies and on budding portions (large arrows) of the mycoplasmas. Plastid (p); cell wall (w). Bar = 500 nm.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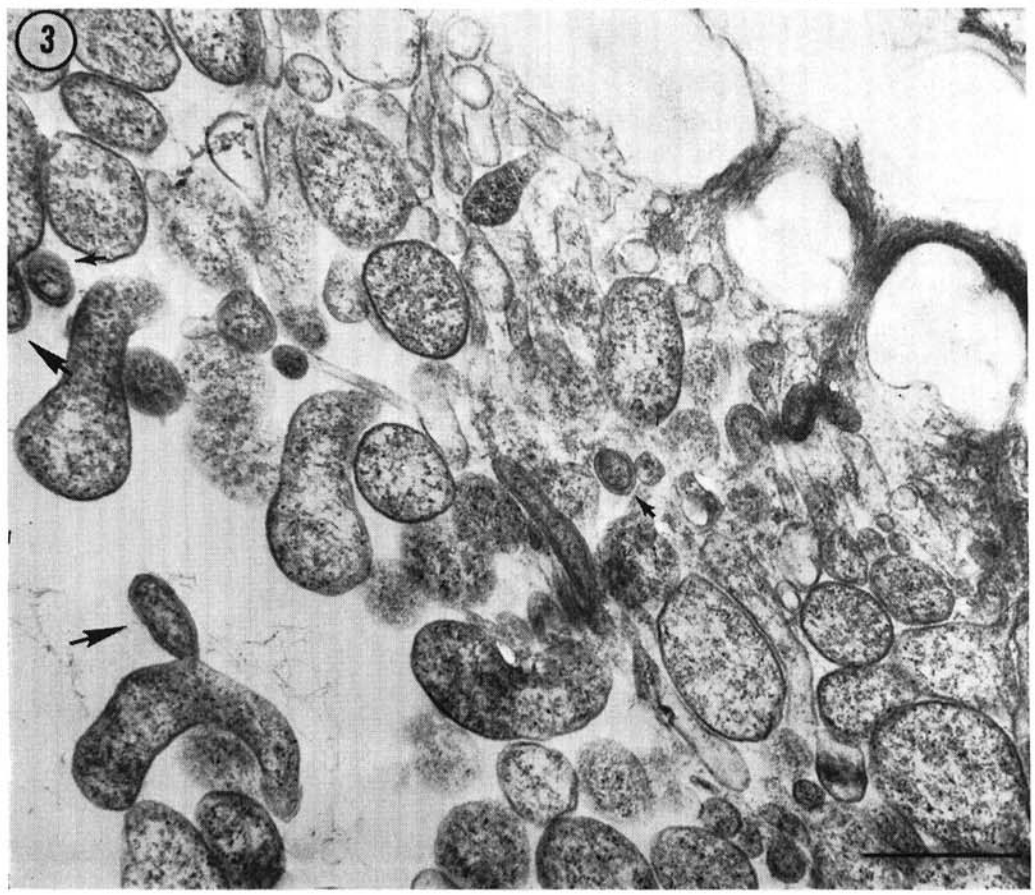
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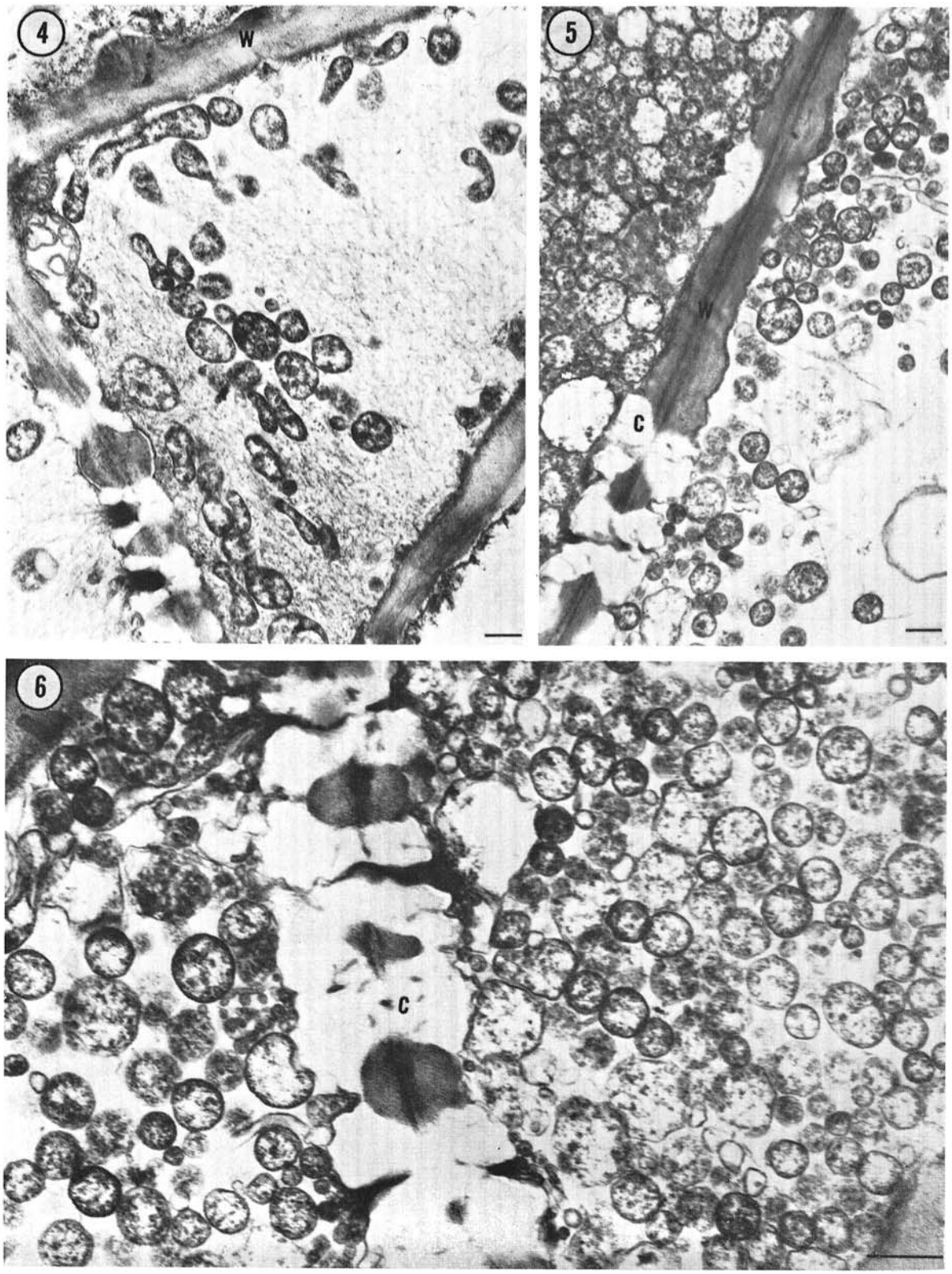

Fig. 4-6. Phloem tissue of *Cuscuta campestris* packed with spherical mycoplasmalike bodies. The dodder was parasitizing yellowed *Vinca rosea*. Callus deposits (c) in phloem sieve pores, cell wall (w). Bar = 500 nm.

not develop yellows symptoms, except for a few plants that were accidentally parasitized by dodder that was growing on adjacent yellowed periwinkle.

Proof of transmissibility of the agent causing the abnormality of periwinkle was determined by cleft-grafting 11 healthy plants with scions from yellowed periwinkle. Ten of the grafted plants developed typical yellows symptoms within 6 months after grafting. In addition, the foliage of healthy carrot (*Daucus carota* L.) plants, when parasitized by *C. subinclusa* or *C. campestris* bridged from yellowed periwinkle, developed symptoms that resembled those described by Kunkel (9, 10) for yellows-infected carrots. These included a stunting and proliferation of secondary shoots from the crown (Fig. 1-E).

Transmission tests were made with sap from leaves of ash and periwinkle with yellows symptoms and from strands of dodder that had been maintained on ash in the field. Crude sap preparations, either combined with sodium thioglycollate (0.02 M) plus sodium diethyldithiocarbamate (0.01 M) in potassium phosphate buffer (0.01 M, pH 7.1), or fractionated on a sephadex gel column, failed to induce disease symptoms when bioassayed on *Nicotiana tabacum* L. 'NN'.

Electron microscopy.—Leaves and washed roots from a healthy and a yellowed ash (greenhouse tree) and from a healthy and a yellowed periwinkle, leaves from a healthy and a yellowed carrot, and segments of *C. campestris* strands grown on a healthy and a yellowed periwinkle were selected for examination. Mycoplasmalike bodies were found in the mature sieve tubes of (i) midveins and petioles of the yellowed ash (Fig. 2, 3), periwinkle, and carrot; (ii) roots of the yellowed ash and periwinkle; and (iii) *C. campestris* that had been parasitizing the yellowed periwinkle (Fig. 4-6). No similar bodies could be found in the parenchyma cells of any of these hosts. The dodder appeared to contain more mycoplasmalike bodies than did the other hosts, since almost every phloem cell was packed with these organisms. This suggests that they grow and reproduce in the dodder. Dale & Kim (1) reported abundant mycoplasmalike bodies in dodder that had been growing on periwinkle infected with aster yellows.

The structures found in diseased tissues were morphologically indistinguishable from the mycoplasmalike bodies associated with other yellows-type diseases of plants (2, 3, 4, 17). Although elongated, ovoid, and filamentous forms were observed, the spherical type appeared to predominate in all four hosts. The dimensions of the spherical bodies varied from 100 to 500 nm in diam; the largest elongated and ovoid bodies measured up to 1 μ in length. The smallest bodies, ca. 100-200 nm in diam, usually appeared densely granular, and may correspond to the elementary body stage (8, 15). The large bodies had the characteristic central fibrillar material surrounded by a more granular peripheral zone. All forms were bounded by a limiting membrane approx 10 nm thick (and presumed to be a unit membrane). Several of the smaller bodies and a few apparently budding forms of the larger bodies appeared to have a double membrane (Fig. 3), the significance of which is now obscure.

No mycoplasmalike bodies were found in healthy tissues. Virus particles were not observed either in the phloem and xylem elements, or in the parenchymatous cells of yellowed tissue.

Mycoplasmalike bodies occurred in such abundance in the leaf blades, petioles, and roots of a yellowed ash, in a periwinkle plant that had been parasitized by dodder transferred from a yellowed ash in the field, and in the dodder itself, that a mycoplasmal etiology is likely for this witches'-broom disease of ash. The apparent absence of virus in infected tissues strengthens this interpretation. Unequivocal proof, however, must await the culture of this microorganism on artificial media so that Koch's postulates can be satisfied.

The increasing number of known hosts of plant mycoplasmas (16) and the identification of insect vectors (12) suggest that this disease of ash could be widespread. Plakidas (13) described a witches'-broom disease of Arizona ash (*F. berlandieriana*). The extent and severity of witches' brooms on ash, particularly in southeastern New York where dieback is severe, are unknown. The significance of this disease in the ash dieback problem, therefore, cannot be ascertained without surveys and additional recovery, infectivity, and vector tests. On the basis of accumulated knowledge of hardwood declines, we can surmise that mycoplasmas, like viruses (5, 6, 7), may constitute yet another incitant among a complex of biotic and abiotic etiologic agents that in combination cause dieback in trees.

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