Mycoplasmalike Organisms, Cause of Lilac Witches'-Broom

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ABSTRACT

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Witches'-brooms developed in *Syringa* × *josiflexa*, *S.* × *prestoniae*, *S. sweginzowii*, and *S. villosa* × *sweginzowii* in the lilac collection at The Morton Arboretum, Lisle, IL. Associated symptoms included proliferation of axillary shoots, shortened internodes, and stunted leaves. Mycoplasmalike organisms (MLO) were detected in phloem sieve tubes of leaves by Dienes' stain and transmission electron microscopy. MLO were also detected in lilacs with symptoms limited to premature growth of buds. Witches'-brooms developed in a lilac infected with MLO by graft transmission.

Many specimens in the collection of 1,200 lilacs at The Morton Arboretum have been deteriorating for more than 10 yr. A wide range of symptoms have been observed, including necrotic blotch, scorch, chlorosis, mottle, mosaic, rolling, stunting, and rugosity of the leaves; premature growth of buds; and dieback of shoots and branches. During an examination of the lilac collection in September 1983, we observed on several cultivars and species the proliferation of axillary shoots (witches'-brooms) and the premature opening and elongation of vegetative and flower buds.

A witches'-broom of lilac has been reported (1). Graft transmission of the infectious agent was achieved, and it was tentatively identified as a virus (2,8). However, virus infection was never confirmed by purification and serology. Mycoplasmalike organisms (MLO), rather than viruses, have been identified as the pathogens responsible for several witches'-broom diseases of trees (10). Symptoms in The Morton Arboretum lilacs resembled those described for tree diseases caused by MLO (10). This report describes lilac witches'-broom (LWB) and presents evidence that MLO cause it.

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MATERIALS AND METHODS

Twenty lilac specimens in the collection and nursery at The Morton Arboretum were selected because of witches'-brooms or premature growth of buds. They were as follows: *Syringa emodi* Don 'Aurea'; *S.* × *josiflexa* Preston 'Royalty'; *S.* × *josikaea* Jacq.; *S.* × *persica* L.; *S.* × *prestoniae* McKelvey 'Elinor,' 'Juliet,' 'Paulina,' and 'Regan'; *S. sweginzowii* Koehne & Lingelsh.; *S. villosa* Vahl; *S. villosa* × *sweginzowii* 'Hedin'; and *S.*

Table 1. Symptoms in Morton Arboretum lilacs selected for study and results of tests for mycoplasmalike organisms (MLO) by Dienes' stain (DS) and transmission electron microscopy (TEM)

			MLO detection[b]		
				TEM	
Lilac identity	Replicate	Symptoms[a]	DS	Dec. 1983	May 1984
Syringa × *josiflexa*					
Royalty	1	++	+	(+)	···
	2	++	+	+	···
	3	++	−	+	···
S. × *prestoniae*					
Elinor		++	+	···	···
Juliet	1	++	+	−	(+)
	2	++	+	−	(+)
Paulina		++	+	···	···
Regan	1	++	+	−	+
	2	++	+	−	+
S. sweginzowii		++	+	···	···
S. villosa × *sweginzowii*					
Hedin		++	+	···	···
S. emodi					
Aurea		+	−	···	···
S. × *josikaea*		+	+	···	···
S. × *persica*		+	+	···	···
S. villosa	1	+	−	···	···
	2	+	−	···	···
S. vulgaris					
Kim	1	+	+	···	···
	2	+	−	···	···
Mme Florent Stepman		+	(+)	···	···
Riet Bruidegom		+	−	···	···
S. sweginzowii		−	−	···	···
S. vulgaris					
Emil Liebig		−	−	···	···
Nadezda		−	−	−	(+)

[a] ++ = Witches'-broom, + = buds swelling or elongating, and − = no symptoms.
[b] + = MLO detected, (+) = MLO possibly present but not distinct, and − = no MLO detected.

vulgaris L. 'Mme Florent Stepman,' 'Kim,' and 'Riet Bruidegom.' The symptoms were observed in September 1983 and 1984. Three apparently healthy lilacs were selected as controls: *S. vulgaris* 'Emil Liebig' and 'Nadezda' and another *S. sweginzowii*.

Dienes' stain. Cuttings of witches'-brooms and nonbroomed shoots were collected from eight lilacs on 9 September and 5 October 1983 and from 15 additional lilacs on 11 September 1984. The cuttings were stored at 4 C until examined. Pieces 3–5 mm long were cut just below the nodes, and longitudinal sections 35–75 μm thick were cut on a freezing microtome. The sections were held 5–7 min in 0.2% Dienes' stain (DS) (4) adjusted to pH 8.3 and mounted in water, then the phloem tissue was examined with a light microscope (× 200–600) for MLO. A lilac was considered infected if MLO were detected in at least three stems. In preliminary work, the DS procedure was practiced with white ash (*Fraxinus americana* L.) and periwinkle (*Catharanthus roseus* (L.) G. Don.) infected with ash MLO (6,9).

Electron microscopy. Cuttings of dormant shoots were harvested on 28 December 1983 from cultivars Royalty, Juliet, and Regan with advanced witches'-broom symptoms and from the healthy cultivar Nadezda. The cuttings were placed in water at room temperature until young shoots and leaves were forced. New shoots were harvested on 8 May from Juliet, Regan, and Nadezda.

Secondary veins, midribs, and petioles from leaves were excised and immediately fixed in a mixture of 2% glutaraldehyde, 1% formaldehyde, 0.5% tannic acid, 0.05% saponin, and 0.001 M $CaCl_3$ in 0.1 M sodium cacodylate buffer (pH 7.2). Primary fixation lasted 3 hr at 21 C under a gentle vacuum. Three changes in fresh fixative solution were made. After three 10-min rinses in 0.1 M sodium cacodylate (800 milliosmols, adjusted with sucrose), the tissue was postfixed with 2% osmium tetroxide in 0.1 M sodium cacodylate buffer, adjusted to 800 milliosmols, for 4 hr in the dark at 4 C. After a thorough rinse in 0.1 M sodium cacodylate buffer followed by three 10-min changes in 0.1 M sodium acetate, the tissue was stained in-block with 1% aqueous uranyl acetate:0.1 M sodium acetate (1:1) and held overnight in the dark at 4 C. The fixed and stained material was washed in 0.1 M sodium acetate (three 10-min changes), dehydrated through an ethyl alcohol graded series and propylene oxide, infiltrated in Polybed 812 (Polysciences, Inc.) embedding medium, and embedded. Sections (60–90 nm) were cut, stained successively in uranyl acetate and lead citrate, and viewed with an RCA EMU-4 transmission electron microscope (TEM) at 100 kV.

Graft transmission. Bud sticks were collected 8 May 1984 from one Royalty lilac with numerous witches'-brooms. Infection was confirmed by DS and TEM. A healthy 4-yr-old Royalty lilac from the Brooklyn Botanic Garden nursery was examined for MLO by DS and grafted with 15 buds and 15 bark patches cut from the bud sticks. The grafted lilac was maintained in partial shade in the greenhouse.

Virus transmission. Cuttings of witches'-brooms were collected from one Juliet lilac. A 15-g sample of shoots plus leaves was indexed for virus by mechanical inoculation of virus indicator plants as described previously (5). A 5-g sample from the grafted Royalty was similarly indexed for virus 8 mo after grafting.

RESULTS

Symptomatology. As summarized in Table 1, 11 lilacs, including cultivars of *S.* × *josiflexa*, *S.* × *prestoniae*, and *S. villosa* × *sweginzowii*, plus *S. sweginzowii*, displayed witches'-brooms in September 1983. These specimens were located mostly in one site in the main collection; a few were in the lilac nursery. Deterioration of these shrubs in both locations had been observed since they were planted in 1979.

Symptoms (Fig. 1) of LWB observed at The Morton Arboretum are as follows: Shoots develop from lateral and apical buds that would normally remain dormant until the following spring. New buds on these shoots also elongate into a second set of axillary shoots. On some stems, the lateral buds remain dormant, but apical growth continues without the formation of a terminal bud. Internode length decreases toward the tips of the shoots, resulting in clumped foliage. The upward angle of axillary shoots is often acute, contributing to the brooming

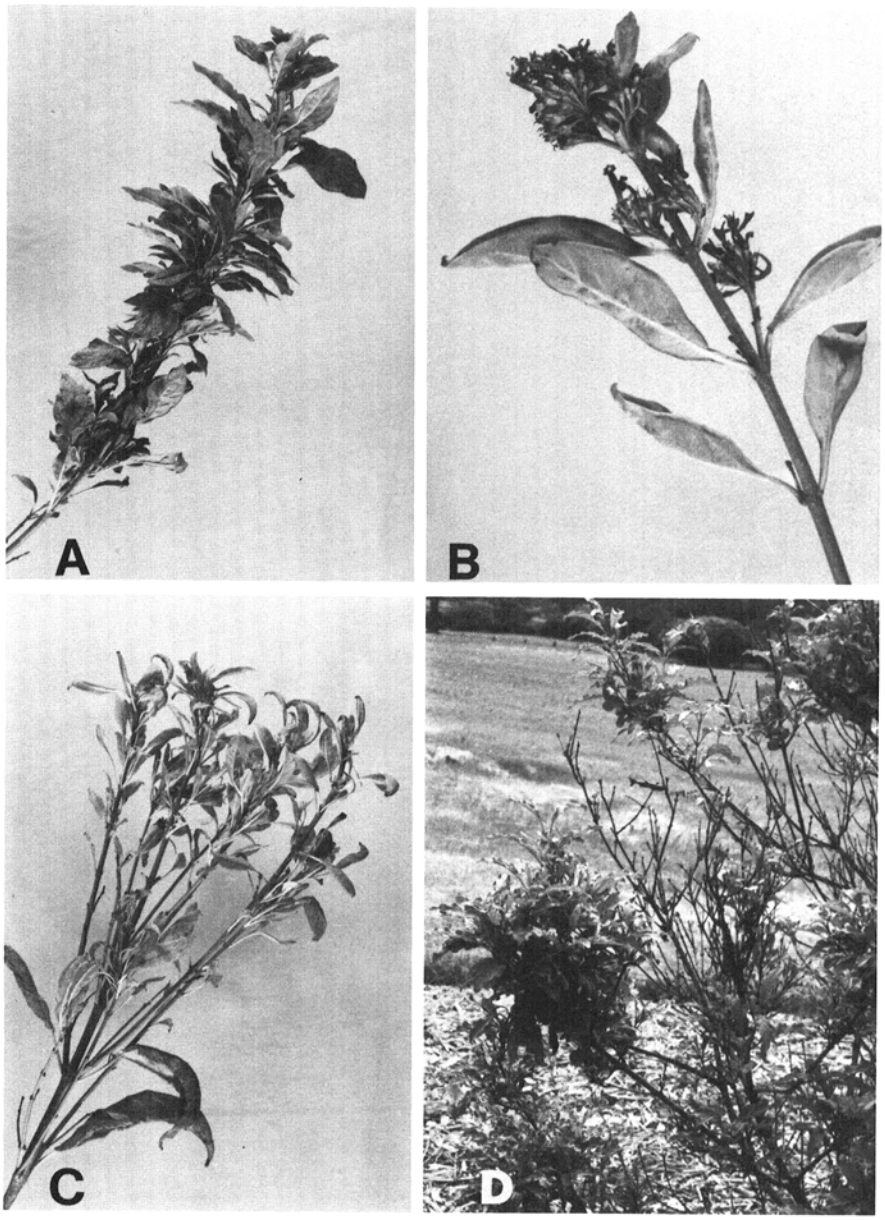

Fig. 1. Symptoms of lilac witches'-broom: **(A)** shoot of Royalty lilac, **(B)** flowers of Juliet lilac in September, **(C)** branch of Juliet showing acute upward angle of shoots, and **(D)** living and dead brooms on Royalty.

appearance of branches. Vegetative buds on older, nonbroomed stems often are stimulated into growth in the autumn. Some witches'-brooms show scorch and dieback during the growing season.

The leaves on witches'-brooms are stunted and elliptical and vary from normal green to chlorotic. Additional symptoms include curling, twisting, puckering, tip scorching, rugosity, and a pink to purple coloration of veins and petioles. Flower buds grow into compact, stunted panicles. Most shrubs with LWB had brooms and healthy-appearing branches intermixed.

Nine lilacs (Table 1) had symptoms limited to the premature swelling or elongation of vegetative or flower buds. These shrubs were located at the same sites as those with witches'-brooms plus a separate site in the main collection that was planted in the mid-1940s.

Dienes' stain. MLO were detected in the phloem sieve-tube elements of 10 of the 11 lilacs with witches'-brooms and in three of the nine with prematurely growing buds (Table 1). No MLO were detected in the three specimens without symptoms.

In longitudinal sections, clumps of MLO appeared medium to dark blue against unstained phloem (Fig. 2A). Clumps were most visible where they abutted unstained sieve plates. Xylem and sclerenchyma cells stained turquoise, which contrasted with the blue-stained MLO in sieve tubes. Numerous MLO clumps were detected in each group of sections from witches'-brooms. They were found less frequently in sections from the three lilacs with prematurely growing buds.

Electron microscopy. MLO were identified by TEM in lilac samples collected December 1983 and May 1984 (Table 1). They were clearly identified in midrib phloem sieve-tube elements of forced leaves from cultivars Regan and Royalty (Figs. 2B and 3). With one exception, samples from these cultivars tested positive by DS (Table 1). Identification by TEM was equivocal in Juliet, Nadezda, and in one Royalty. Lilac MLO (Figs. 2B and 3) varied in size from 0.2 to 1 μm and had trilamellar unit membranes, DNA-like fibrils, and ribosomes, all characteristic of plant-pathogenic MLO (3).

Graft transmission. No MLO were detected in the healthy Royalty lilac before grafting. The grafted lilac began showing symptoms after 5 mo. Two apical and three lateral nongrafted 1984 buds had produced shoots 1–15 cm long that developed stunted light green to chlorotic leaves. New axillary buds on the forced shoots also elongated into stunted, misshapen leaves. Six months after grafting, 45 nongrafted vegetative buds had produced shoots that resembled those seen on the infected Royalty shrubs in The Morton Arboretum. Thirteen 1984 stems showed dieback (1–12 cm).

The presence of MLO in the phloem of shoots from the grafted lilac was confirmed by DS and TEM.

A shoot from the grafted lilac was tested for the presence of spiroplasmas by C. J. Chang (Department of Plant Pathology, University of Georgia, Experiment). Extracts of leaf and stem tissues were cultivated on isolation media as described by Liao and Chen (7). No spiroplasmas were detected in the cultures.

Virus transmission. No symptoms indicating virus infection developed on the virus indicator plants. No viruslike particles were observed in the phloem sieve-tube elements during examination of tissue samples by TEM.

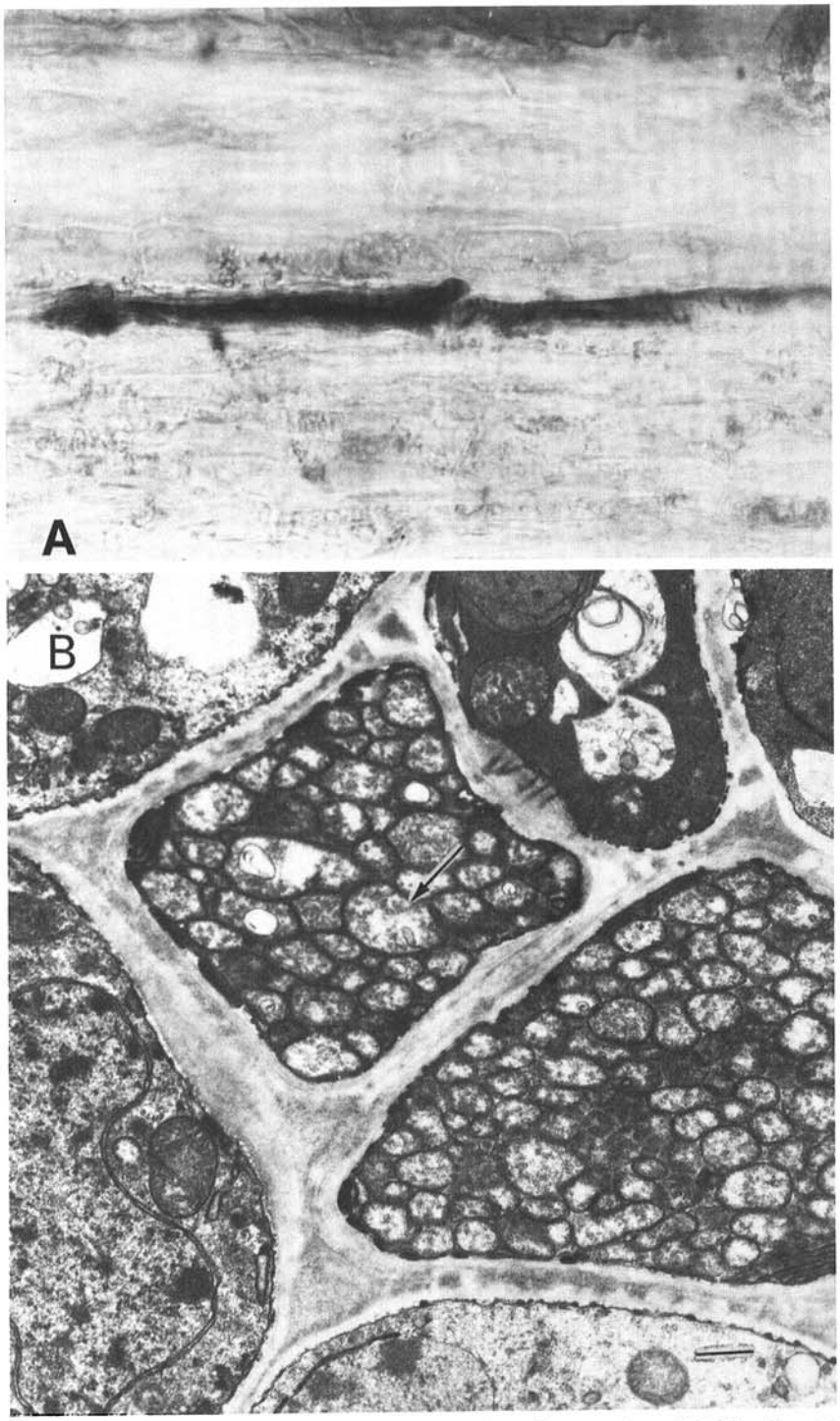

Fig. 2. (A) Clumps of dark (from Dienes' stain) mycoplasmalike organisms (MLO) in sieve-tube elements of a shoot from infected Royalty lilac (×600). **(B)** MLO (arrow) in sieve-tube element of midrib from leaf of infected Regan lilac. Scale bar = 0.5 μm.

DISCUSSION

We conclude that the likely causal agent of LWB at The Morton Arboretum is an MLO. This is based on the occurrence of witches'-brooms typical of those known to be caused by MLO, the

identification of MLO in the phloem of symptomatic lilac leaves, and the inducement of MLO infection and typical symptoms by graft transmission. It is likely that MLO causing LWB were introduced to the arboretum through infected stock from an outside source.

Davis and Whitcomb (3) use the term "yellows disease" to designate plant diseases caused by MLO. Because yellowing of foliage is not a prevalent symptom of LWB, we have chosen to maintain the original name, lilac witches'-broom, first used by Brierley (1). Brooms may not be the only indicator of infection by MLO. From the DS results, but unconfirmed by TEM, lilacs with symptoms limited to the premature swelling or elongation of buds also can be infected. Because lilac buds tend to open during unusually warm weather in autumn, or after defoliation during the growing season, this symptom is not a reliable indicator of infection by MLO.

Electron microscopy confirmed the presence of MLO in the phloem sieve-tube elements and corroborated the legitimacy of the DS test for diagnosing MLO infection of lilacs. The successful use of DS for detecting MLO in trees has been reported (9). The difficulty of locating MLO in plant tissue by TEM, and the probable variability in MLO titer, could account for the instances where DS results were not corroborated by TEM. Alternatively, one Royalty lilac had distinct witches'-brooms and MLO were identified by TEM, yet they were not detected by DS. A negative stain test, therefore, is not proof of the absence of MLO.

MLO infection of lilacs is significant for several reasons. In addition to disfiguring woody hosts, MLO cause reductions in stem and root growth, disrupt the time and quality of flowering, and cause dieback and mortality (10). Infection by MLO can predispose woody hosts to climatic stresses. White ash infected with MLO (9) and citrus infected with spiroplasma (10) were reported to have subnormal cold hardiness. Several of The Morton Arboretum lilacs with LWB in September 1983, but not adjacent healthy lilacs, died during the severe winter of 1983–1984. Because MLO are transmissible by natural and artificial grafts, through cuttings, and by phloem- and xylem-feeding insects, LWB has the potential to become widespread. We have observed witches'-brooms in other lilac collections. More information is needed on the impact of this disease on lilac, susceptible and resistant taxa, insect vectors, and its geographical range.

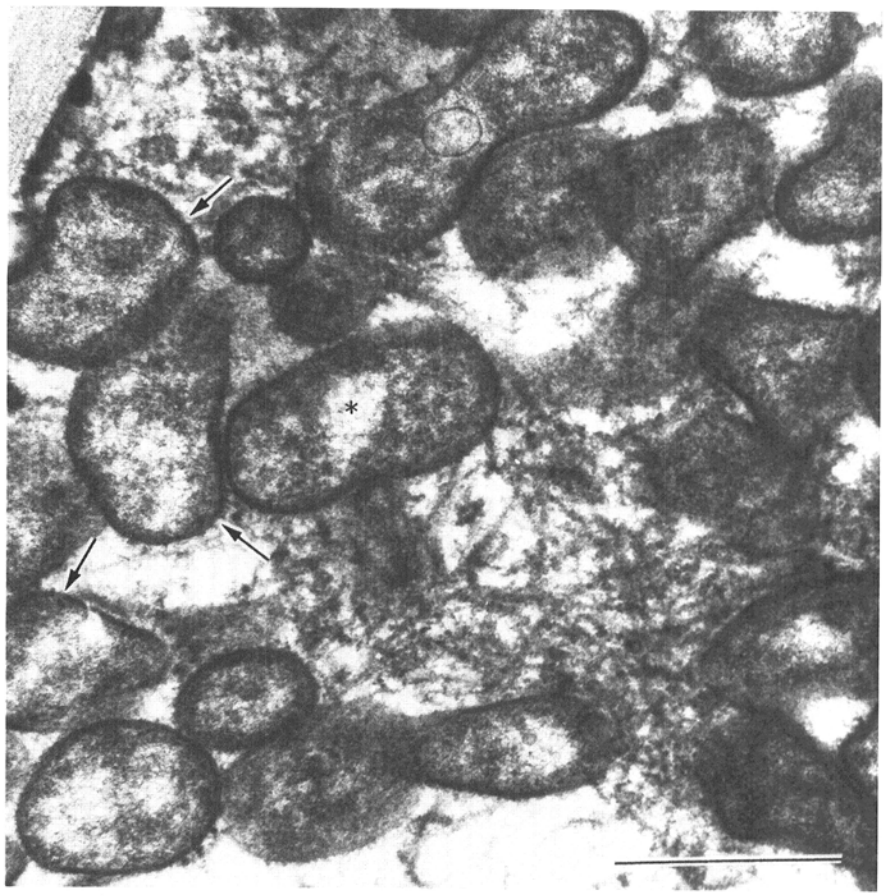

Fig. 3. Mycoplasmalike organisms in sieve-tube element of Royalty lilac. Typical trilamellar membranes (arrows) and DNA-fibrillar network (*) are visible. Scale bar = 0.5μm.

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