Susceptibility of Lilacs to Mycoplasmalike Organisms[1]

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Abstract

The cause of lilac witches'-broom has been identified as a mycoplasmalike organism (MLO). From field symptoms and the detection of MLO in the phloem by fluorescence microscopy, *Syringa vulgaris* cultivars were identified as susceptible but more tolerant of infection than non-*vulgaris* lilacs. The MLO were graft-transmissible but not seed-transmissible. Mycoplasmal infection has been identified in the following lilac species and hybrids: *S.* × *diversifolia*, *S.* × *henryi*, *S. henryi* × *tomentella*, *S.* × *josiflexa*, *S. josikaea*, *S. julianae*, *S. komarowii*, *S. laciniata*, *S. meyeri*, *S. microphylla*, *S.* × *nanceiana*, *S. oblata* var. *dilatata*, *S.* × *persica*, *S.* × *prestoniae*, *S. sweginzowii*, *S. villosa*, *S. villosa* × *sweginzowii*, *S. vulgaris*, and *S. yunnanensis*.

Index words: lilac, *Syringa*, witches'-broom

Introduction

A witches'-broom of lilac was reported in 1951 (1), and the cause was presumed to be a virus (2). In 1985, mycoplasmalike organisms (MLO), rather than a virus, were identified as the cause of lilac witches'-broom in *Syringa* × *josiflexa*, *S.* × *prestoniae*, *S. sweginzowii*, *S. villosa* × *sweginzowii*, *S. josikaea*, *S.* × *persica*, and one cultivar of *S. vulgaris* (6).

We have continued to study lilac collections in the eastern U.S. and Canada to learn more about the range and severity of this disease. The witches'-broom symptoms (proliferation of axillary shoots, shortened internodes, stunted leaves) have not been observed in cultivars of *S. vulgaris*, even when interplanted with non-*vulgaris* lilacs showing severe brooming. Because *S. vulgaris* cultivars, including most of the Lemoine or French hybrids, are widely grown, we conducted a survey to determine their susceptibility to MLO. This report presents evidence for mycoplasmal infection of *S. vulgaris* cultivars, and up-dates our compilation of the host range of MLO in the genus *Syringa*.

Materials and Methods

Lilacs examined. Cultivars of *S. vulgaris* located in three arboreta were examined for disease symptoms, and tissue samples were harvested for microscopic detection of MLO. Witches'-broom had been identified in non-*vulgaris* lilacs in the three sites (5). The Centennial Lilac Garden, Niagra Falls, Ontario, Canada was examined in June, 1987. Sixteen cultivars were selected for study, all of which were adjacent to non-*vulgaris* lilacs showing witches'-broom symptoms. The display lilacs at the Arnold Arboretum, Jamaica Plain, Massachusetts were examined in October, 1988. Thirteen cultivars of *S. vulgaris* were selected, all showing scattered twig dieback and chlorotic or undersized foliage. The lilac arboretum in Highland Park, Rochester, New York was examined in May, 1987. Twenty four cultivars were selected, many of which showed abnormal twig growth and scattered twig dieback. The same 24 cultivars were examined and sampled in November, 1988.

Sampling and DAPI fluorescence tests. Three current-year shoots and three roots were cut from each shrub (shears dipped in 70% isopropyl alcohol between shrubs), placed in plastic bags, and later held at 4°C (39°F). One segment, 0.5–1 cm (0.2–0.39 in), was cut from each shoot and root sample. Shoot segments included a node. Root segments were 1–3 mm (0.04–0.12 in) diam. Segments that could not be processed immediately were held in a fixative consisting of 2.5% gluteraldehyde in 0.1M PO_4 buffer, pH 7. The controls were 2-yr old healthy lilacs grown from seed.

The shoot and root segments were mounted in Tissue Tek O.C.T. Compound (Miles Scientific, Naperville, Illinois) on the stage of a freezing microtome. Longitudinal sections to include secondary phloem were cut at 10–15μ for shoots and 5–10μ for roots. The sections were rinsed in 0.1 M PO_4 buffer at pH 7, stained for 20 min in aqueous DAPI (4′,6-diamidino-2-phenylindole•2HCl; 2HC1; Sigma Chemical Co., St. Louis, Missouri) at 0.4 μg/ml, and rinsed in buffer. For each lilac, five shoot sections and five root sections were mounted in 50% aqueous Karo white corn syrup on microslides. The stained sections were examined at 200X and 400X with an Olympus Model BH2-RFL epi-fluorescence microscope with exciter filter UG1 and barrier filter L420.

The use of DAPI for detecting MLO in lilacs has been reported (5). To confirm the accuracy of this method for the work with *S. vulgaris* cultivars, a shoot sample from *S. vulgaris* 'Montaigne', located in Highland Park and testing positive for MLO by DAPI, was examined by transmission electron microscopy, courtesy of Dr. Tseh An Chen, Department of Plant Pathology, Rutgers University. A shoot sample from a healthy lilac, which tested negative for MLO by DAPI, was included as a control.

Graft transmission. Fourteen 6-yr old *S. vulgaris* lilacs, one 4-yr old *S.* × *josiflexa* 'Royalty', and one 6-yr old *S.* × *josiflexa* 'Anna Amhoff', were grafted with buds (T-budding) and bark patches which had been excised from MLO-infected brooms. The brooms were cut from *S.* × *josiflexa* 'Royalty' located at the Arnold Arboretum. Each lilac received 15 bud grafts and 15 bark patches, spread evenly among the stems. The presence of MLO in the scion-

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wood, and their absence in the stock lilacs before grafting, were confirmed by Dienes' stain (3). The grafted lilacs were maintained in a screened greenhouse and observed for symptoms.

Seed transmission. Seed was collected in September, 1986 and 1987 from the following lilacs at the Arnold Arboretum: *S.* × *henryi* 'Lutece', *S.* × *prestoniae* 'Maybelle Farnum', *S.* × *josiflexa* 'Royalty', and *S. villosa*. The seed-source lilacs showed abundant witches'-brooms, and MLO infection of the shrubs was confirmed by DAPI tests. The seed was stratified for 3 months at 5°C (39°F). After germination, 25 seedlings of each species were transplanted to a soil-less mix in 4-in pots and held in a screened greenhouse. In January, 1989, ten of the lilacs from seed of each species were selected for DAPI tests, as described above.

Results and Discussion

Detection of MLO. The DAPI binds with mycoplasmal DNA and fluoresces blue-white under UV radiation. A DAPI test was rated positive when the abnormal fluorescence could be detected clearly in at least three locations in phloem sieve-tube elements within each group of five sections. Fluorescence denoting MLO was not observed in the control lilacs. The MLO were identified by electron microscopy in sieve-tube elements of *S. vulgaris* 'Montaigne', but not in the control lilac. This confirmed the accuracy of the DAPI test. Figure 1 shows MLO in phloem sieve-tube elements of *S.* × *prestoniae* 'Regan'.

The DAPI tests revealed mycoplasmal infection in the *S. vulgaris* cultivars. Based on their detection in either shoots or roots (Tables 1–3), MLO were identified in 74.5% (38/51) of the selected cultivars.

The percentage of cultivars identified as infected by MLO was not greater at the Centennial Lilac Garden (64.3%) than at the Arnold Arboretum (69.2%) or Highland Park (83.3%). This was noteworthy because in the Centennial Lilac Garden, the lilacs selected for study were in juxtaposition with non-*vulgaris* lilacs bearing abundant witches'-brooms. These site conditions would favor the transmission of MLO through root grafts and by phloem feeding insects.

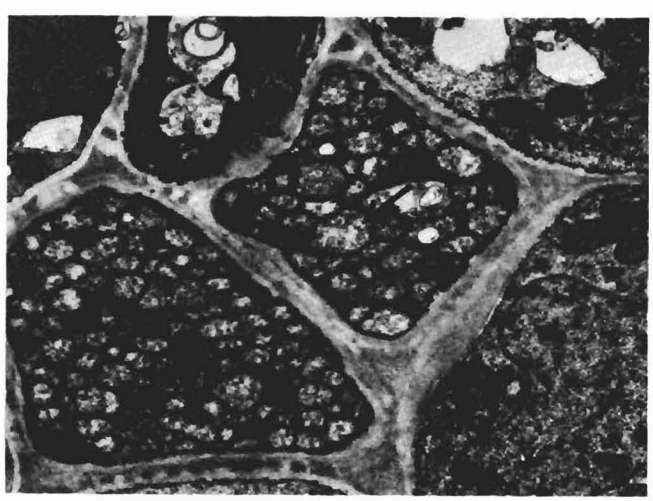

Fig. 1. Mycoplasmalike organisms (arrow) in sieve-tube elements of midrib from leaf of infected 'Regan' lilac. Scale bar = 0.5um. (Photograph by John D. Castello).

Table 1. Symptoms and results of DAPI fluorescence tests for detection of mycoplasmalike organisms in selected *Syringa vulgaris* cultivars in the Centennial Lilac Garden, Niagra Falls, Ontario.

Cultivars	Symptoms[z]	DAPI fluorescence tests[y]	
		Shoots	Roots
Congo	2	−	−
Edith Cavell	1	+	−
Edith Cavell	2	+	−
Frank Patterson	3	+	+
Georges Bellair	1	−	−
Krasavitsa Moskvy	1	−	−
Lucie Baltet	2,3	+	+
Mme. Florent Stepman	2	+	−
Mme. Lemoine	2	−	−
President Poincare	2,3	+	−
President Viger	2	+	+
Princess Clementine	1	−	−
unidentified	2,3	+	−
unidentified	1	+	−

[z]1 = healthy, no symptoms; 2 = scattered twig dieback, 3 = leaves undersized, sometimes with chlorosis.

[y]Presence (+) or absence (−) of fluorescing MLO in phloem sieve-tube elements.

For some woody hosts, MLO are more likely to be detected in roots than in shoots (11). The percentage of sampled lilacs in which MLO were detected only in shoots was 31.4% (16/51), only in roots, 3.9% (2/51); and in both shoots and roots, 39.2% (20/51). Therefore, to detect MLO in lilacs, examination of both shoots and roots is recommended.

Symptoms in relation to infection. Symptoms in MLO-infected lilacs are not limited to witches'-brooms (Fig. 2). Current-year buds breaking dormancy prematurely during the summer or fall, shoot growth from adventitious buds in older wood, and abnormalities in twig growth (Fig. 3) have been associated with MLO infection in non-*vulgaris* lilacs (5, 6).

Table 2. Results of DAPI fluorescence tests for detection of mycoplasmalike organisms in selected *Syringa vulgaris* cultivars with decline symptoms[z] in the Arnold Arboretum, Jamaica Plain, MA.

Cultivars	DAPI fluorescence tests[y]	
	Shoots	Roots
Capitaine Perrault	+	+
Carmine	+	−
Charles Joly	+	+
Ekenholm	−	−
Gaudichaud	+	+
Mlle. Fernande Viger	+	−
Mme. Catherine Bruchet	−	−
Mme. Florent Stepman	+	+
Marlyensis Pallida	−	−
Nana	+	−
Paul Hariot	+	−
Vergissmeinnicht	−	−
Verschaffeltii	+	−

[z]Poor growth, chlorosis and downwrd curl of leaves, scattered twig dieback.

[y]Presence (+) or absence (−) of fluorescing MLO in phloem sieve-tube elements.

Table 3. Symptoms and results of DAPI fluorescence tests for detection of mycoplasmalike organisms in selected *S. vulgaris* cultivars in Highland Park, Rochester, NY.

Cultivars	Symptoms[z]	DAPI fluorescence tests[y]	
		Shoots	Roots
Capitaine Perrault	2,3,4	+	+
Col. Wm. R. Plum	3,4	+	−
De Miribel	3,4	−	−
Geheimrat Heyder	3	+	+
Grand-Duc Constantin	2,3	+	+
Hugo Koster	3,	+	−
Jessie Gardner	2,3	+	+
Joan Dunbar	2,3	+	−
Maurice de Vilmorin	4	+	+
Mauve Mist	1	−	−
Mauve Mist	2,3	+	+
Miss Ellen Willmott	2,3	+	+
Miss Ellen Willmott	4	+	−
Mme. Henri Guillaud	2,3	−	−
Montaigne	2,3,4	+	+
Patrick Henry	2,3	−	+
Princess Camille de Rohan	2,3	+	+
Prof. E.H. Wilson	4	+	+
Sarah Sands	2,3	−	+
Souvenir de Claudius Graindorge	2,3	+	+
Souvenir de Henri Simon	3,4	+	−
Splendor	2,3	−	−
Triste Barbaro	2,3	+	+
Victor Lemoine	1	+	+

[z]1 = healthy, no symptoms; 2 = stunted twigs in contorted and zig-zag pattern, often in lower or interior part of shrub, 3 = scattered twig dieback, 4 = current-year buds breaking dormancy, or shoots growing from adventitious buds.

[y]Presence (+) or absence (−) of fluorescing MLO in phloem sieve-tube elements; data for shoots based on two samplings: May, 1987 and November, 1989.

The MLO were detected in 79.5% (35/44) of the lilacs with symptoms (Tables 1–3). Two symptoms appeared to be diagnostic for infection. In Highland Park, MLO were identified in 12 of 14 lilacs with stunted twigs in contorted and zig-zag growth patterns. This irregular twig growth has been observed on MLO-infected *S.* × *prestoniae* cultivars (5). Infection also was associated with lilacs bearing current-year buds which broke dormancy prematurely and initiated growth in the fall (Table 3). This symptom is not always diagnostic for infection, as lilac buds occasionally expand prematurely after prolonged warm periods late in the growing season. Witches'-brooms were not observed on any *S. vulgaris* cultivars. The dieback and foliar symptoms (Tables 1–3) were interpreted as diagnostic for a number of possible stresses, including scale and borer infestations and drought.

The MLO were identified in 3 of 7 lilacs rated healthy. These results corroborate previous DAPI and Dienes' stain tests (unpublished), in which MLO could be detected in healthy-appearing *S. vulgaris* lilacs.

Graft transmission. The MLO were transmitted by bud or bark patch grafting, as confirmed by DAPI tests. The symptoms induced by graft-transmitted MLO were consistent with field symptoms of infected lilacs. Six months after grafting, both 'Anna Amhoff' and 'Royalty' showed vegetative buds breaking dormancy prematurely. This was followed by broom formation, twig dieback, and plant mortality within another 11 months. Transmission occurred in one *S. vulgaris* lilac. By 10 months after grafting, 'Mme. Lemoine' showed vegetative buds breaking dormancy prematurely. Infection was detected in only one major branch, and the symptoms were milder: occasional leaf chlorosis and curl, vegetative and flower buds breaking dormancy prematurely, and shoot tip dieback.

Fig. 2. Witches'-brooms on infected 'Royalty' lilac.

Fig. 3. Stunted and bunchy twig growth, with scattered twig dieback, on infected 'Royalty' lilac.

Seed transmission. Symptoms indicative of MLO infection did not occur in any of the 100 1 and 2-yr old lilacs which were raised from seed from infected lilacs. No MLO were detected in the roots or shoots of the 40 seedling lilacs which were selected for testing by DAPI. These results are consistent with reports that MLO are not transmitted through seed (11).

Host range. Lilac taxa in which the witches'-broom disease has been identified (as of December 1988) are compiled in Table 4. This information is based on field symptoms plus the detection of MLO in shoot or root tissues by Dienes' stain or DAPI tests. Some of this information has been reported (5).

Late blooming lilacs (4) appear to be especially susceptible (Table 4). Among the hybrid late blooming lilacs, susceptibility occurs often in lilacs with *josikaea* or *villosa* lineage, e.g.,: *S.* × *henryi* (*S. josikaea* × *S. villosa*), *S.* × *josiflexa* (*S. josikaea* × *S. reflexa*), *S.* × *nanceiana* (*S.* × *henryi* × *S. sweginzowii*), and *S.* × *prestoniae* (*S. reflexa* × *S. villosa*).

No symptoms indicative of infection by MLO have been observed in *S.* × *hyacinthiflora* early blooming cultivars, but extensive DAPI tests have not been made. More research is necessary before lilacs with apparent resistance can be recommended with confidence.

Table 4. Lilac taxa in which the witches'-broom disease has been identified by symptoms and by the detection of mycoplasmalike organisms in the phloem of shoots or roots by Dienes' stain or the DAPI fluorescence test.

Early Blooming Lilacs
Syringa oblata var. *dilatata* (Nakai) Rehd.

Midseason Blooming Lilacs
S. × *diversifolia* Rehd. (*S. pinnatifolia* × *S. oblata*) 'Nouveau'
S. josikaea Jacq. f. ex Reichb. 'Eximia'
S. julianae Schneid.
S. laciniata Mill.
S. meyeri Schneid.
S. microphylla Diels 'Superba'
S. × *persica* L. (pro sp.) (*S.* ? × *S. laciniata*)
S. vulgaris L. cvs. Aurea, Bleuatre, Boule Azuree, Captitaine Perrault, Carmine, Charles Joly, Colbert, Col. Wm. R. Plum, Dr. Charles Jacobs, Edith Cavell, Fountain, Frank Patterson, Gaudichaud, Geheimrat Heyder, Grand-Duc Constantin, Hugo Koster, Hunting Tower, Jessie Gardner, Joan Dunbar, Kim, Le Gaulois, Lucie Baltet, Maurice de Vilmorin, Mauve Mist, Miss Ellen Willmott, Mlle. Fernande Viger, Mme. Florent Stepman, Montaigne, Nana, Patrick Henry, Paul Hariot, Petersons, Pinkie, President Poincare, President Viger, Princess Camille de Rohan, Prof. E.H. Wilson, Sarah Sands, Souvenir de Claudius Graindorge, Souvenir de Henri Simon, Sulte, Triste Barbaro, Verschaffeltii, Vestale, Victor Lemoine

Late Blooming Lilacs
S. × *henryi* Schneid. (*S. josikaea* × *S. villosa*) 'Lutece'
S. × *henryi* × *S. tomentella* Bur. & Franch. 'Prairial'
S. × *josiflexa* Preston ex Pringle (*S. josikaea* × *S. reflexa* Schneid.) cvs. Anna Amhoff, Elaine, Enid, Guinevere, Royalty
S. komarowii Schneid.
S. × *nanceiana* McKelvey (*S.* × *henryi* × *S. sweginzowii*) cvs. Floreal, Rutilant
S. × *prestoniae* McKelvey (*S. reflexa* × *S. villosa*) cvs. Alexander's Aristocrat, Alice, Calpurnia, Charmian, Constance, Coral, Dawn, Desdemona, Dorcas, Elinor, Francisca, Isabella, James Macfarlane, Juliet, Lavinia, Maybelle Farnum, Miranda, Nellie Bean, Olivia, Paulina, Portia, Regan, Romeo, Silvia, Ursula, Virgilia
S. sweginzowii Koehne & Lingelsh.
S. villosa Vahl
S. villosa × *sweginzowii* 'Hedin'
S. yunnanensis Franch.

Lilac collections in which the disease has been identified are as follows: Arnold Arboretum, Jamaica Plain, Massachusetts; Boerner Botanical Gardens, Hales Corners, Wisconsin; Dominion Arboretum, Ottawa, Ontario, Canada; Highland Park, Rochester, New York; Holden Arboretum, Mentor, Ohio; Lilacea Park, Lombard, Illinois; Morton Arboretum, Lisle, Illinois; Centennial Lilac Garden, Niagra Falls, Ontario, Canada; and Royal Botanical Gardens, Hamilton, Ontario, Canada.

Significance of disease. Mycoplasmal pathogens are harmful to woody landscape plants in several ways (12). They disfigure their hosts, cause chlorosis in foliage, reduce leaf, shoot, and root growth; lessen the quality of flowers, disrupt the normal time of flowering, and induce dieback and, sometimes, plant mortality. These impairments all occur with lilac witches'-broom. Infection by MLO can predispose woody hosts to non-biological stresses. Infected white ash were reported to have subnormal cold hardiness (10). We have observed the death of MLO-infected lilacs following a severe winter (6).

Because MLO are transmissible by vegetative propagation and by phloem and xylem feeding insects (12), these pathogens can become widespread. There is circumstantial evidence (8) that the same or related strains of MLO can infect hosts in the genera *Syringa* and *Fraxinus*. By increasing the host range, there is a greater chance for the acquisition and spread of the lilac MLO by insect vectors.

Significance to the Nursery Industry

The mycoplasmal pathogen causing lilac witches'-broom has a wide host range in the genus *Syringa*. The *S. vulgaris* lilacs are susceptible, but they react to infection with less severe symptoms than non-*vulgaris* lilacs. The pathogen is graft-transmissible, but not seed-transmissible. Field symptoms can be diagnostic for infection by MLO, but infection can occur in lilacs showing no overt symptoms.

Our research has been on lilacs in arboreta, so the extent of mycoplasmal infection in nursery lilacs is unknown. We know, however, that the disease can be spread within collections by vegetative propagation, and among collections by the exchange of infected plant material (7). Spread by insects is likely, as leafhoppers and other phloem feeding insects are known to be transmitters of MLO.

Until more is known about the control of MLO diseases in woody plants, our recommendations to nurserymen are as follows: (a) Inspect lilac nursery stock for disease symptoms. Watch for brooming, excessive thin and stunted twigs, stunted twigs in contorted and zig-zig patterns, current-year buds breaking dormancy late in the growing season, and shoot growth from adventitious buds on older wood. (b) Rogue out and destroy lilacs suspected of being infected. (c) Do not propagate vegetatively from lilacs suspected of being infected. The lilac MLO cannot be transmitted through seed. (d) Dip tools in 70% isopropyl alcohol (rubbing alcohol) after pruning infected lilacs. (e) Accept only healthy non-*vulgaris* cultivars, especially those late blooming lilacs known to be severely affected (Table 4).

Lilac propagation by tissue culture is becoming increasingly important (9). Contamination of explants by MLO may have to be monitored to assure the propagation of

disease-free stock. The selection of healthy lilacs as sources of explants is made more difficult by the fact that lilacs can be infected by MLO, yet appear healthy. We are investigating the production of MLO-free lilacs by shoot apex culture.

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