Association of a Mycoplasmalike Organism with a Disease of Annual Statice in Michigan

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ABSTRACT

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Mycoplasmalike organisms (MLOs) were found in phloem cells of ultrathin sections of stems, leaves, and pedicels of annual statice (*Limonium sinuatum*) showing the following symptoms: yellowing and malformation of young leaves, leaf reddening in older rosettes, bunching of flower stalks, phyllody, and other abnormal flower development. Similar organisms were not found in phloem cells of symptomless plants. The MLOs were transmitted to healthy statice, celery, and aster by the leafhopper *Macrosteles fascifrons*. Symptoms in statice resembled those observed in the field. This is the first report of an MLO associated with a disease of annual statice in Michigan.

In the summers of 1981 and 1982, a disease of unknown etiology was discovered in annual statice, *Limonium sinuatum* Mill. Annual statice is a horticultural crop grown mostly for the commercial florist industry for both fresh and dried uses. The disease occurred at several locations in mid-Michigan, accounting for losses as high as 80% in some fields. Symptoms included yellowing, veinclearing, bunching and malformation of young leaves, reddening of leaves in the basal rosette, stunting, abnormal flower stalk development resulting in a proliferation of shortened stalks resembling a witches' broom, phyllody, and other abnormal flower development including bud blast, reduced flower size, abnormal shape and color, and failure of flowers to open (Fig. 1). The purpose of this investigation was to determine the cause of the disease.

MATERIALS AND METHODS

Diseased and healthy plants of annual statice were collected in September 1981 and July and August 1982 near Grand Rapids, MI. Tissues from 10 diseased and four apparently healthy (symptomless) plants were collected each year for transmission electron microscopy (TEM), virus indexing, and isolation studies. For TEM, four 1-mm segments were cut from stems, leaves, and pedicels of both diseased and symptomless plants and immediately immersed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, on ice. After 2–4 hr, samples were washed in buffer, postfixed 2 hr in 1% buffered OsO_4, dehydrated in a graded series of ethanol, and embedded in a mixture of Mollenhauers No. 1 (Epon-Araldite) and ERL epoxy resin (2). Monitor sections (1 μm) were cut, stained with 2% toluidine blue O, and examined with a Wild light microscope. Ultrathin sections were stained with 2% uranyl acetate and lead citrate (6) and examined in a Philips 201 transmission electron microscope.

Tissues were prepared for isolation studies by surface-sterilizing in a 1:10 dilution of commercial Clorox (0.525% sodium hypochlorite) for 5 min and plating portions of stems, leaves, pedicels, and flowers on both potato-dextrose agar (PDA) and nutrient agar (NA). Agar plates were incubated at 22 C and examined daily for 2 wk for bacteria and/or fungi.

Leaves and flower stalks were assayed for virus by mechanical inoculation of Carborundum-dusted indicator plants with sap from diseased plants. Infected tissue was ground in a mortar and pestle with the addition of enough 0.1 M phosphate buffer, pH 7.2, to promote grinding. The following indicator plants were used: *Chenopodium quinoa, Nicotiana tabacum* var. *xanthi*-nc, *N. tabacum* var. *turkish, Phaseolus vulgaris* var. *pinto*, and *Datura stramonium*.

Leafhoppers were used in an attempt to transmit the disease to healthy plants. Fourth and fifth instar nymphs of *Macrosteles fascifrons* (Stål) were caged for 7 days on the same field-collected diseased plants used for TEM and then transferred to barley (*Hordeum vulgare* L.) for 14 days. Individual leafhoppers were caged on six to eight healthy seedlings each of annual statice, aster (*Callistephus chinensis* L.), and celery (*Apium graveolens* L.) for 7 days or until death. Both inoculum and test plants were kept at 22–25 C and 16 hr of light throughout the experiment. As a control, leafhoppers from the same colony were caged on healthy statice and transferred as described. Tissue samples from test plants showing disease symptoms were processed as described and examined by TEM.

RESULTS

No bacterium or fungus was consistently isolated from either diseased or healthy tissue. No symptoms developed on any of the mechanically inoculated virus indicator plants and no virus or viruslike particles were observed when diseased

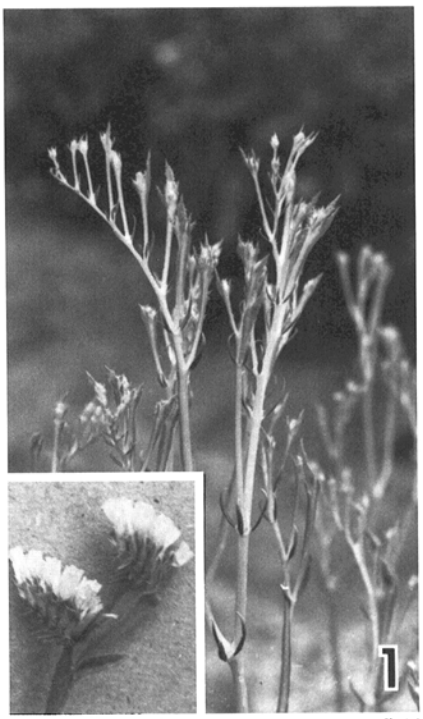

Fig. 1. Statice (*Limonium sinuatum*) field symptoms: abnormal flower development including phyllody and failure of flowers to open. **(Inset)** Normal flower development.

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statice tissues were examined with the transmission electron microscope.

Examination of monitor (1-μm) sections revealed mycoplasmalike organisms (MLO) in sieve-tube elements from stems, leaves, and pedicels of diseased plants. The shape of the MLOs was polymorphic. No spiroplasmas were noted.

TEM also revealed numerous MLOs in the sieve-tube elements of the phloem in all pedicel, stem, and leaf sections from all diseased plants collected in both 1981 and 1982 (Fig. 2). Only sieve-tube cells of diseased plants appeared to contain the organisms. No MLOs were found in companion cells or parenchyma. The MLOs ranged from 0.2 to 0.4 μm in diameter, were bound by a unit membrane, and again were noted to be spherical to polymorphic in shape when examined in serial sections. Tissue from the symptomless plants did not contain the organism.

In the leafhopper transmission studies, none of the control plants developed symptoms (Fig. 3A) nor were MLOs found in their phloem tissue. Statice, aster, and celery test plants used in the transmission studies, however, developed symptoms 2–3 wk after infected leafhoppers were removed or had died. Individual *M. fascifrons* survived poorly on statice seedlings, all dying before the 7-day inoculation feeding ended, but still apparently transmitted the MLOs. Statice seedlings showed veinclearing, prolific crown growth, and reduced leaf blade formation (Fig. 3B). Aster seedlings showed typical aster yellows (AY) symptoms including veinclearing, stunting, floral virescence, and general chlorosis (Fig. 3C). Celery seedlings also showed typical AY symptoms including twisted stalk growth from the crown, purple-brown stalk color, and leaf chlorosis. All three symptomatic seedling species showed MLOs in their phloem tissue when examined ultrastructurally. The MLOs were limited to sieve tubes and were polymorphic in shape, as in field-collected plants.

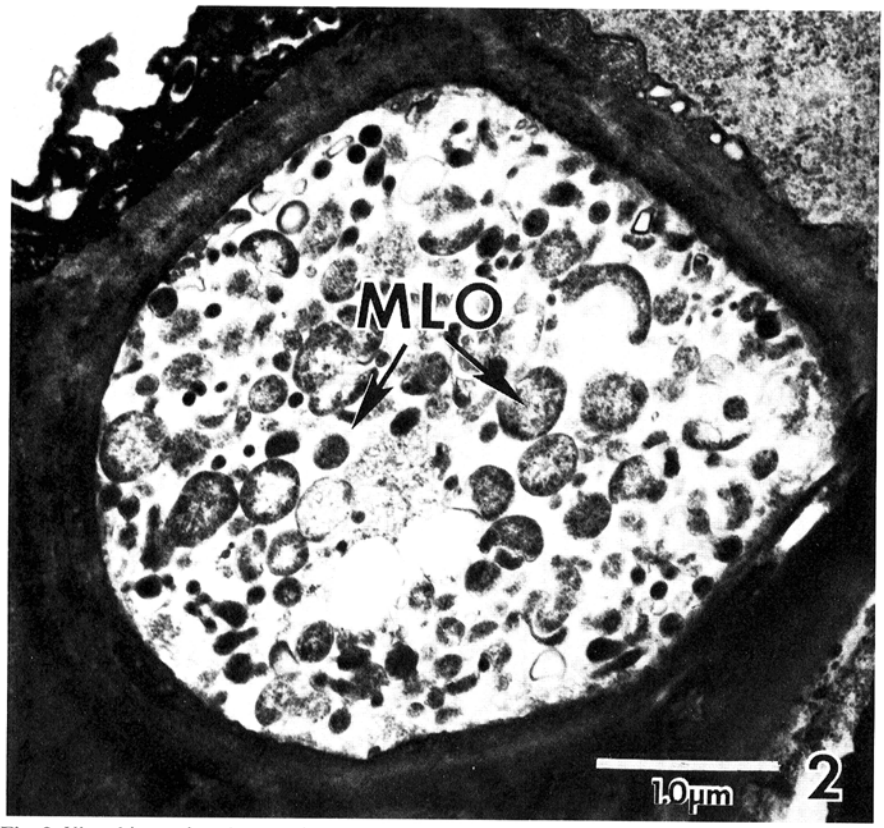

Fig. 2. Ultrathin section through flower stalk of diseased statice. Phloem sieve-tube cell filled with mycoplasmalike organisms.

DISCUSSION

In California, AY has been reported in perennial statice, *Limonium* spp. (7), and turnip mosaic virus has been reported in *L. perezii* and *L. sinatum* (5), but we were unable to induce symptoms of turnip mosaic virus in *N. tabacum* or *C. quinoa* when these assay plants were mechanically inoculated with sap from diseased statice. Other diseases of unknown etiology on statice have been reported in Florida (1). Symptoms in these diseases, such as reddening of basal foliage, were similar to those we observed in 1981 and 1982 in Michigan; however, the causal agent(s) for the disease(s) of statice in Florida was not determined although symptoms were indicative of the AY MLO.

Muller et al (4) noted MLOs in the sieve tubes of *Limonium* and other ornamentals showing flower phyllody. Both the symptoms on *Limonium* and the morphology of the MLO appeared similar to that reported in this paper, but no information on transmission by leafhoppers was reported in that study.

Characteristics of yellows diseases, particularly AY, include such symptoms as stunting, veinclearing, prolific crown growth, sterility, witches' brooms, and the presence of phloem-limited MLOs (3). In this study, the symptoms and presence of MLOs, similar to aster

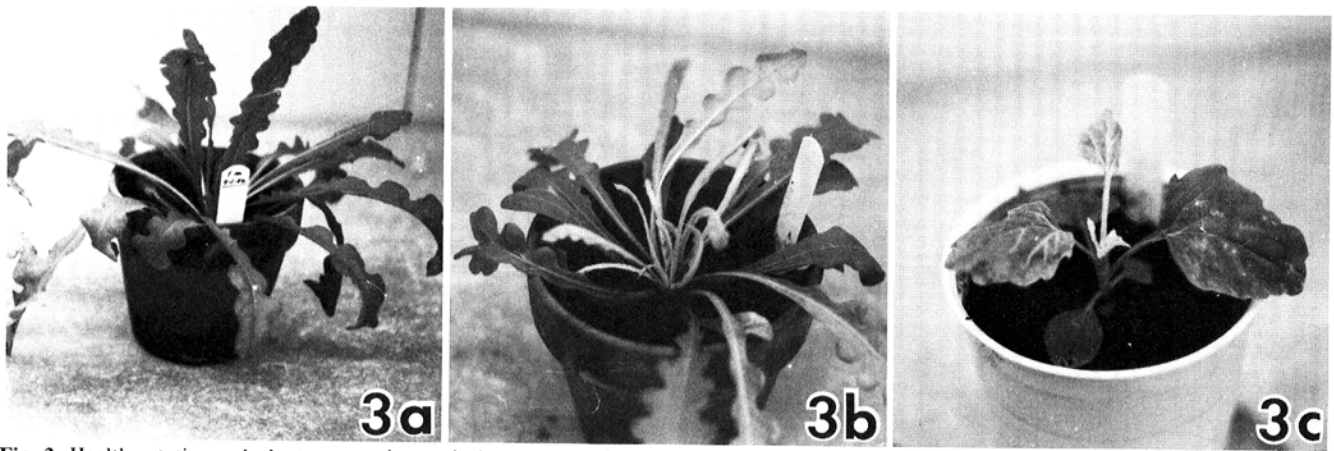

Fig. 3. Healthy statice and plants expressing typical symptoms after transmission of mycoplasmalike organisms by the leafhopper, *Macrosteles fascifrons*: **(A)** healthy statice, **(B)** diseased statice, and **(C)** diseased aster.

yellows, were noted for both diseased plants from the field and for seedlings infected by the leafhoppers. Symptoms were more severe in the infected seedlings than in field-collected plants and seedlings generally did not recover. It is not known when statice plants in the field become infected, although these studies indicate that infection by MLOs might occur later in the season and/or might be of lower titer in the plants because symptoms were not as severe in the plants collected in the field as in test plants grown in the greenhouse and exposed to infected *M. fascifrons*.

The symptoms noted in the field in Michigan, the presence of MLOs in phloem cells in diseased tissue, and the ability to transmit the MLOs to healthy plants using *M. fascifrons*, a known AY vector, with the subsequent development of symptoms, are substantive evidence that the MLO, perhaps AY, played a primary role in this disease of annual statice. This is the first report of a probable mycoplasma-associated disease of commercially grown annual statice in Michigan.

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