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PERIWINKLE WITCHES' BROOM DISEASE IN SOUTH FLORIDA[1]

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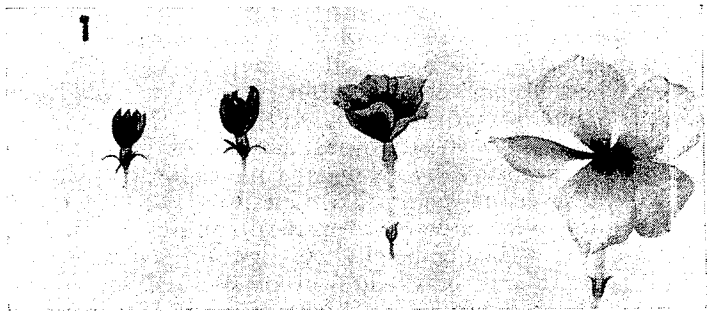
Abstract. **A witches' broom disease of Madagascar periwinkle has been observed in South Florida. Mycoplasmalike organisms are readily found in the sieve tubes of affected tissues. Symptoms after graft transmission are virescence and phyllody followed by proliferation of branches and dwarfing of foliage. The initial witches' broom effect comes from branches arising from the modified stamens of phylloid flowers. At elevated temperatures, the witches' brooms do not develop and leaves and flowers are of normal size and color, although flower proliferation may occur.**

A witches' broom disease of Madagascar periwinkle, *Catharanthus roseus* (L.) G. Don (Vinca rosea), has been found in naturalized plants growing wild in various areas of southern Florida. Symptoms of the disease have been seen in all months of the year, and up to 50% of the plants in some locations have been observed to be affected. In nature, the witches' broom symptoms are found on one or several branches only of any particular plant. The remaining branches of the plants appear normal and systemically affected plants have not been seen.

Graft inoculated periwinkles grown in pots do become systemically affected and all branches develop symptoms. This paper describes the association of mycoplasmalike organisms (MLO) with this disease, as well as symptomatology as observed in the field and in the greenhouse. Symptom development is contrasted to that of aster yellows in periwinkle, another MLO-associated disease.

Methods and Results

Symptomatology. Witches' broom diseased periwinkles found in nature have only a few branch tips showing typical symptoms of branch proliferation, reduction in foliar size, and occasional phylloid or virescent flowers. Affected branches do not exhibit hypertrophy and generally do not flower. Healthy periwinkles graft-inoculated with diseased scions from the field develop symptoms of virescence within 2 to 4 weeks in a greenhouse when average temperatures are below 86°F (30°C). As symptoms develop, new flowers become green (virescent) (Fig. 1). With further intensification of the symptoms, the parts of subsequent flowers become leaf-like or phylloid. Ultimately, the phylloid flowers become difficult to recognize as the branch proliferation producing the witches' broom appearance results from the conversion of the stamens and ovary to leafy structures (Fig. 2)



Figs. 1-4. Symptoms of periwinkle witches' broom disease. 1. Virescence showing greening and dwarfing of petals, normal flower on right. 2. Phyllody or conversion of floral parts to vegetative organs. Note leaf-like shape of converted petals and emergence of leafy shoots from the base of the corrolla. 3. Branch symptoms of aster yellows (left), and witches' broom (middle) diseases of periwinkle. Healthy plant on right. 4. Proliferation of Virescent flowers. Arrows denote sepals. Normal flower on left.

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and eventually, to stems (Fig. 3). Often, three to five branches arising from a region of the stem with very short internodes indicates the conversion of reproductive to vegetative structures during disease development. The breaking of dormancy of lateral buds adds to the witches' broom effect. The branches of the witches' broom are thin and have short internodes. They do not exhibit the hypertrophy evident in stems of aster yellows affected periwinkles (Fig. 3). Also, the witches' broom effect in aster yellows diseased periwinkles arises primarily from lateral branch proliferation, rather than from phylloid flowers, and the branches have a spindly appearance due to elongated internodes.

Temperature response. To determine the effect of temperature on symptom expression, young periwinkle plants of ca. 4 inches height were graft-inoculated with either aster yellows or witches' broom-affected scions and placed in lighted incubators. Lights were warm-white and red fluorescent tubes and photoperiod was 16 hrs. per day. Temperatures tested were 80, 86 and 90 ± 1.0°F and checked against a calibrated wide-range mercury thermometer.

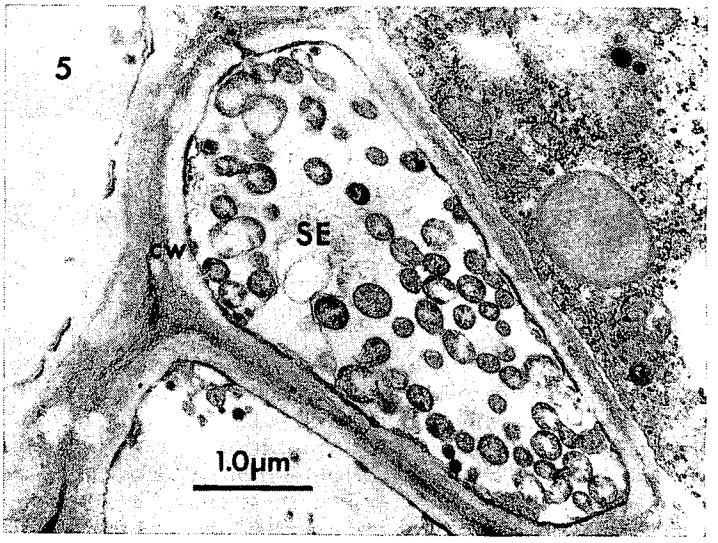
Symptoms for both diseases appeared 14 days after grafting for both witches' broom and aster yellows at 80 and 86°F. No symptoms developed at a constant temperature of 92°F (35°C) for either disease. Initial symptoms at 80 and 86°F were virescence for witches' broom disease and breaking of dormancy of lateral buds for aster yellows. Subsequently, the virescence symptoms of witches' broom intensified with new growth until only phylloid flowers were produced. Later, witches' brooms appeared from the greatly reduced phylloid flowers.

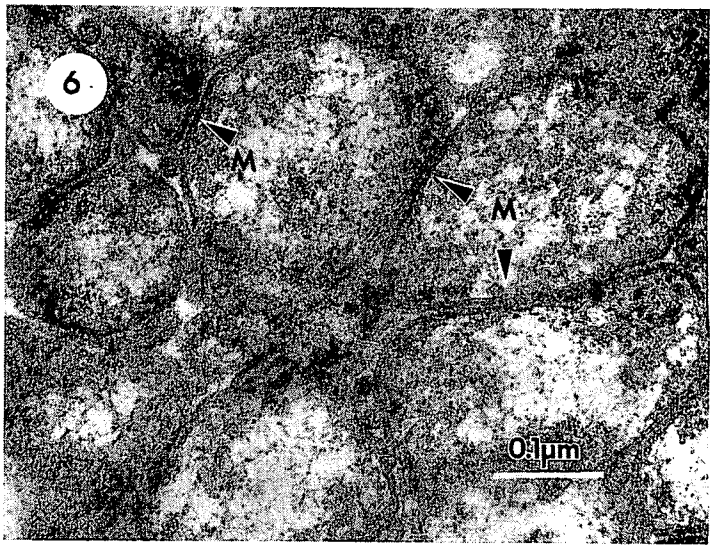
In aster yellows disease, several virescent flowers were produced in the first week or two of symptom development. Subsequently, no floral organs were produced, stems became greatly swollen, and leaves were reduced in size with swollen petioles and midribs devoid of chlorophyll. Ultimately, the breaking of lateral buds resulted in witches' brooms with long spindly stems and greatly reduced leaves.

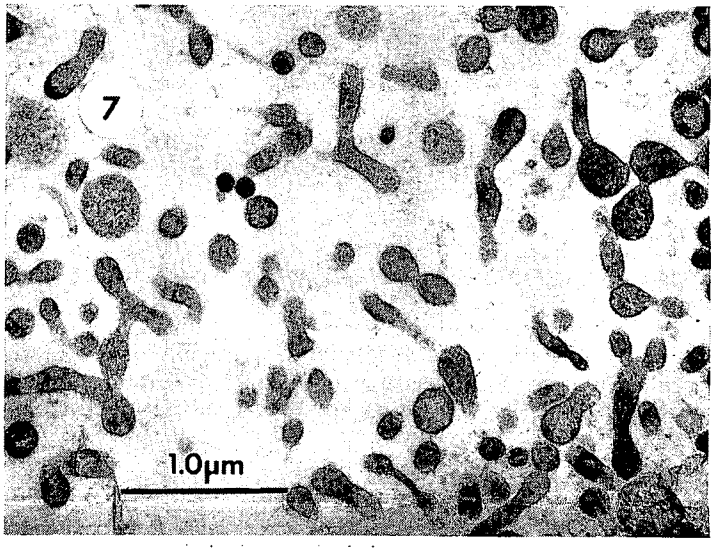
Witches' broom-affected plants placed in a non-ventilated greenhouse in summer where temperatures averaged 92°F produced virescent flowers without developing phyllody or witches' broom symptoms. Often, at these elevated temperatures, a flower proliferation occurred in which virescent flowers developed from the centers of other virescent flowers (Fig. 4). Occasionally a flower proliferation occurred in which flowers of normal size and color developed from the centers of otherwise normal appearing flowers.

Electron microscopy. Petiole samples from affected plants were trimmed to ca. 1mm sections in cold fixative and held 18 hours in 0.1 M collidine-buffered 2% gluteraldehyde-2% paraformaldehyde fixative at pH 7.4. Specimens were post-fixed for 6 hours in 0.1 M collidine-buffered 2% osmium tetroxide followed by 0.5% aqueous uranyl acetate for 18 hours, all at 65°F (8°C). After dehydration in a graded ethanol-acetone series specimens were embedded in Spurr plastic, and ultra-thin sections taken for examination by the transmission electron microscope.

Typical, polymorphic mycoplasmalike organisms were present in sieve elements of affected plants. Phloem of healthy plants contained no such bodies. Bodies were 0.2 to 0.8 μm in diameter, contained ribosomes and DNA, and were bounded only by a single unit membrane (Figs. 5 & 6). Elongate to filamentous forms were often observed along with rounded forms within the same sieve element (Fig. 7). Examination of thick sections (0.3 μm) did not reveal any mycoplasmas of helical morphology, but did indicate the presence of branched, filamentous forms and occasional beaded chain forms of MLO.







Figs. 5-7. Electron micrographs of mycoplasmalike organisms (MLO) within sieve elements of witches' broom diseased periwinkle. 5. MLO in sieve element (SE), cell wall (CW). 6. High magnification electron micrograph of MLO showing unit membrane (M) containing fibrillar DNA network and ribosomes. 7. Elongate to filamentous forms of MLO in sieve element.

Discussion

The witches' broom disease of periwinkle in south Florida is associated with a polymorphic mycoplasmalike organism. The presence of virescence and phyllody along with the witches' broom, yellowing, and stunting symptoms are typical of a yellows group disease (3). Since branch proliferation is the predominant symptom, the disease has been termed witches' broom.

The relationship of periwinkle witches' broom to other plant diseases associated with mycoplasmalike organisms is not known, although the symptoms are distinct from those of aster yellows disease in periwinkle. Periwinkle witches' broom has been noted well outside of active lethal yellowing foci so it is highly doubtful that it is caused by the MLO associated with lethal yellowing disease of coconut palm (4, 5). Since no helical mycoplasmas were seen in infected tissue, it is unlikely that the pathogen is either *Spiroplasma citri*, cause of citrus stubborn disease, or the corn stunt spiroplasma. In any case, no helical mycoplasmas have been shown to produce virescence symptoms (1).

Periwinkle has been demonstrated to be a host for numerous plant mycoplasmas and MLO (1, 3), some of which can be differentiated on the basis of symptomatology. However, without side by side comparison it cannot be determined if periwinkle witches' broom is the same as the vinca virescence disease reported from *California* (2). Ultimately, the identification and separation of different mycoplasmal pathogens of plants will be possible when these agents are isolated in pure culture. Until such time, detailed taxonomic comparison of these pathogens must be based on symptomatology, host range, and vector relationships.

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CHEMICAL CONTROL OF MYROTHECIUM DISEASE OF GLOXINIA[1]

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Abstract. **The virulence of five isolates of** Myrothecium roridum **Tode ex Fr. was demonstrated on 'Improved Red Vevlet' gloxinia** (Sinningia speciosa **(Lodd.) Hiern.). Soil drenches of iprodione 50W at 1.0 g/liter gave effective, nonphytotoxic control of the leaf spot and crown rot phases of the disease. The leaf spot and crown rot were also controlled with soil drenches of captafol 4F at 7.1 ml/liter and 14.2 ml/liter, captan 50W at 4.8 g/liter and zinc ion maneb complex 80W at 3.6 g/liter. These treatments, however, were phytotoxic. Soil drenches of benomyl 50W at 0.6 g/liter and 1.2 g/liter, ethazole + thiophanate-methyl 40W at 0.7 g/liter and 1.3 g/liter, iprodione 50W at 0.5 g/liter, and RH 2161 2EC at 0.6 ml/liter and 1.2 ml/liter were not phytotoxic but were ineffective in controlling the disease.** In vitro **resistance of a gloxinia isolate of** M. roridum **to benomyl and ethazole + thiophanate-methyl fungicides was demonstrated.**

Myrothecium roridum Tode ex Fr. incites a collar rot and leaf spot of gloxinia (*Sinningia speciosa* (Lodd.) Hiern.) (3). During hot, damp weather the disease can be a serious threat to plant production. All ages of gloxinia are susceptible (3). The disease is most conspicuous when they are about 5 months old and beginning to flower. Because of the substantial investment of time and money required before plants reach this age, preventive measures against the disease are essential.

The present study was initiated to determine the virulence of 5 isolates of *M. roridum* from 5 sources on gloxinia, to assess disease control with chemicals, and to evaluate the resistance of a gloxinia isolate of *M. roridum* to fungicides containing benomyl or thiophanate-methyl.

Materials and Methods

Difco potato dextrose agar (PDA) cultures of each *M. roridum* isolate were grown under constant fluorescent light at 28°C for 7 days before storage as 5 mm discs under mineral oil in constant darkness at 10°C.

Experiment 1. Virulence of M. roridum isolates on gloxinia. The virulence of 5 isolates of *M. roridum* was determined on 'Improved Red Velvet' gloxinia. Inoculum was produced by growing each isolate on PDA under constant, cool-white fluorescent light at 28°C for 10 days. Conidia were harvested by adding deionized H_2O (DW) to the surface of cultures and rubbing with a rubber policeman. Conidial suspensions of each isolate were filtered through 2 layers of cheesecloth and adjusted to $1x10^6$ conidia/ml. These suspensions were used promptly to inoculate 4-week old seedlings.

Seedlings to be inoculated were removed from flats and large portions of their root systems were exposed. Groups of 12 seedlings were immersed in a conidial suspension or DW (control) for 5 minutes. The plants were then planted singly in 10 cm diam plastic pots containing a soil mix consisting of Florida peat, sand, vermiculite, and perlite (5:3:3:1 v/v ratio) supplemented with dolomite, hydrated lime, superphosphate, and a trace element mix. The soil mix (used for all subsequent plant experiments) was amended with 2.5 cc 14-14-14 Osmocote® time release fertilizer per pot and supplemented with water soluble fertilizer as needed. All seedlings were watered immediately after planting. Treatments were replicated 12 times in a randomized complete block design. All plants were rated for

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