Natural Infection of *Salvia uliginosa* with Cucumber Mosaic Cucumovirus

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Abstract. *Salvia uliginosa* Benth. plants, in an experimental planting of *Salvia* species, exhibited virus-like symptoms of chlorotic line patterns and ringspots. The suspect virus was mechanically transmitted to several experimental hosts and was identified as cucumber mosaic cucumovirus (CMV) based on dsRNA gel patterns, positive reaction with CMV antiserum, and particle morphology as observed by transmission electron microscopy.

Annual salvia, *Salvia splendens* Sell ex Roem. & Schult., is well known and widely planted in southern landscapes while perennial salvias are less well known, but becoming more popular (Clebsch, 1997). Twenty-five perennial salvia species and cultivars are undergoing evaluation at Burden Research Plantation in Baton Rouge for their potential use in landscape plantings. During the 1996 disease evaluations, virus-like symptoms, consisting of chlorotic line patterns and, occasionally, ringspots, were observed in *Salvia uliginosa* (bog salvia). No other salvia species showed these symptoms in the trial planting. The objective of this study was to identify the virus causing the disease symptoms.

Materials and Methods

Cuttings from salvia plants with and without virus-like symptoms (Fig. 1) were collected and rooted and the plants were maintained in a greenhouse. Inoculum for virus transmission tests was prepared by grinding leaves from plants showing symptoms in a mortar in cold buffer [0.01 M sodium phosphate, plus 0.01 M sodium diethyl dithiocarbamate (DIECA), pH 7.0], together with a small amount of 600-mesh silicon carbide abrasive. Inoculum was rubbed on leaves of test plants with the end of a pestle; then inoculated leaves were rinsed with tap water. Plants were held in a greenhouse maintained at 24 °C and observed for symptom development. Species used for virus transmission tests included cucumber (*Cucumis sativus* L., cv. Poinsett), cowpea [*Vigna unguiculata* (L.) Walp., cv. Purple Hull], four-o'clock (*Mirabilis jalapa* L.), tobacco (*Nicotiana benthamiana* Domin,

and *N. clevelandii* A. Gray), and bog salvia.

Agar double-diffusion serology tests were performed in plastic petri dishes using 0.9% sodium chloride, 0.5% Ionagar No. 2 (Colab Laboratories, Inc., Chicago Heights, Ill.), and 0.02% sodium azide per 100 mL deionized water. Sap extracts from healthy and suspected virus-infected salvia were diluted with 2 mL sodium phosphate buffer (0.01 M, pH 7.0) per gram of tissue and placed in peripheral agar wells. Cucumber mosaic virus (CMV) antiserum was placed in center wells. Sap extracts from healthy and infected salvia were negatively stained with 2% uranyl acetate (pH 6.8) and viewed with a JEOL 100 CX transmission electron microscope (Joel USA, Inc., Peabody, Mass.) to determine virus particle morphology. Double-stranded RNA was extracted from 3.5-g leaf samples using a modi-

fication of the Morris and Dodds CF-11 cellulose column chromatography (Morris and Dodds, 1979; Valverde et al., 1990). Extracts were analyzed for dsRNA by electrophoresis in 6% polyacrylamide gels at 100 V for 2.5 h. A satellite-containing isolate of tobacco mosaic virus (TMV) and identified cucumber mosaic virus isolates were included in tests as controls.

Results and Discussion

A virus was successfully transmitted mechanically from *S. uliginosa* plants showing virus-like symptoms to five indicator plant species. Symptoms on indicator plants included line patterns, ringspots, mosaic, leaf distortion, and local necrotic lesions (Table 1). The virus was transmitted back to healthy *S. uliginosa* plants and produced line pattern symptoms like those originally observed. Virus-infected stock *S. uliginosa* plants occasionally showed leaf distortion symptoms on new growth in the greenhouse, but this symptom was not observed on field-grown plants. Agar double-diffusion tests were positive and specific precipitin bands were formed between wells that contained CMV antiserum and those that contained sap from salvia samples showing symptoms. No precipitin bands formed with sap extracts from healthy salvia. Electron microscopy revealed the presence of spherical particles ≈30 nm in diameter in virus-infected salvia samples. Gels of dsRNA extracts from salvia plants with virus symptoms showed banding patterns similar to those for known strains of CMV (Fig. 2). Based on the evidence presented, we conclude that the *S. uliginosa* plants showing virus-like symptoms were infected with a satellite-RNA-carrying strain of cucumber mosaic cucumovirus.

Fig. 1. *Salvia uliginosa* leaves. (Top) healthy; (bottom) infected with cucumber mosaic cucumovirus.

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Table 1. Experimental hosts and their symptoms after inoculation with the suspect virus from *Salvia uliginosa*.

Host plant	Symptoms
Cucumber	Severe chlorotic ring spotting
Cowpea	Local necrotic lesions and systemic mosaic
Four-o'clock	Mosaic, leaf distortion
Tobacco	Mosaic, leaf distortion
Bog salvia	Chlorotic line patterns

Cucumber mosaic virus is an economically important virus that infects ~500 plant species in 67 families on a worldwide basis (Francki and Habili, 1996; Horvath, 1980). The virus is known to infect a large number of herbaceous and woody ornamental plants that include nandina (*Nandina domestica* Thunb.), ajuga (*Ajuga reptans* L.), euonymus [*Euonymus japonica* var. *microphylla* (Thunb.) H. Jaeg.], four-o'clock, chrysanthemum (*Dendranthema × grandiflora* Kitam.), and various lilies (*Lilium* sp.) (Barnett and Baxter, 1973; Francki and Habili, 1996; Smith, 1972). Coleus mosaic, first described in 1945, was recently shown to be caused by CMV in Louisiana (Holcomb and Valverde, 1991). Cucumber mosaic virus reportedly occurs in the following salvia species outside of the United States: *S. lyrata* L., *S. nemorosa* L., *S. pratensis* L., and *S. splendens*

Fig. 2. Agarose gel electrophoresis of dsRNAs from different cucumber mosaic virus (CMV) isolates. Lane 1, TMV plus satellite; lane 2, CMV-malva isolate; lane 3, CMV-Apios isolate; lane 4, CMV-cowpea isolate; and lane 5, CMV-salvia isolate.

Sellow ex Roem. & Schult. (Debrot et al. 1974; Horvath, 1980). This is the first report of the occurrence of CMV in *S. uliginosa*. Long-term effects of virus infection on this plant are not known at this time.

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