Identification of Two Sweet Potato Feathery Mottle Virus Strains in North Carolina

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ABSTRACT

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A survey of commercial sweet potato fields showed two widely distributed strains of sweet potato feathery mottle virus (FMV). One strain caused only foliar symptoms in sweet potato (*Ipomoea batatas* 'Jersey'); the other, RC-FMV, caused the russet crack disease on Jersey roots. The two strains were further distinguished by foliar symptoms produced in *I. nil* and *I. purpurea* and by local lesion development on *Chenopodium amaranticolor* or *C. quinoa* by RC-FMV. The FMV strain did not infect *Chenopodium* spp. Foliar symptoms in Jersey and Jewel cultivars and other *Ipomoea* spp. consisted of chlorotic vein banding and chlorotic spotting for both strains. The two strains were serologically related in microprecipitin tests, had thermal inactivation points of 60–65 C, dilution end points of 10^{-3} to 10^{-4}, and longevities in sap of less than 1 day. These studies support previous findings that the russet crack virus is a strain of FMV.

Observations of North Carolina sweet potato (*Ipomoea batatas* (L.) Lam.) fields during the summer of 1976 showed that nearly all plants had foliar symptoms previously attributed to virus diseases (6,8,12). These symptoms included chlorotic spots, some with purple borders, and chlorotic vein banding and feathering.

Virus diseases of sweet potato have been described and named on the basis of foliar or root symptoms, but investigations of the causal agents have been impeded because of their narrow host range, absence of differential hosts, and the apparent labile nature of these viruses. Inability to separate possible mixed infections has left in doubt the uniqueness of many of these diseases; the problem of sweet potato virus nomenclature has been reviewed elsewhere (5).

In research to identify viruses infecting sweet potato in North Carolina, sweet potato feathery mottle virus (FMV) was initially identified (11); variable symptoms observed in *I. nil* 'Scarlet O'Hara' were eliminated by serial aphid transfers. Symptom variability suggested the presence of additional viruses or strains of FMV in source plants. Isolates obtained from South Carolina exhibited properties consistent with those reported here (4).

The primary objectives of this study were to determine the nature of the symptom variability observed during isolations from naturally infected sweet potato and the distribution of the virus or strains in the commercial sweet potato production areas of North Carolina.

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MATERIALS AND METHODS

Isolation. Previous studies (1,11) showed 0.05 M potassium phosphate, pH 7.2, containing 0.1 M sodium diethyldithiocarbamate to be effective for FMV transmission from sweet potato. These studies used only symptomatic leaf tissue as a source; symptoms on field-grown sweet potatoes are expressed primarily on mature, fully expanded leaves that presumably contain high concentrations of inhibitors.

Additional trials were made to select the optimum tissue source for isolation from sweet potato. Cultivars Porto Rico and Jewel infected with FMV were used as sources. Tissue was assayed from four stages of developing leaves beginning from the distal end of the vine: 1) the two youngest leaves, 2) oldest symptomless leaves, 3) symptomatic leaves, and 4) young symptomatic leaves produced from lateral buds near the proximal end. Each sample was triturated in 10 volumes of buffer and rubbed onto 20 *I. nil* seedlings dusted with 600-mesh Carborundum.

Survey. Mechanical isolations were made from Jewel sweet potato plants collected in 18 fields in seven counties (Columbus, Johnston, Nash, Sampson, Wake, Wayne, and Wilson) of eastern North Carolina during August 1977. Vine cuttings with symptomatic leaves were taken from 10 plants in each field and were rooted and maintained in 35 × 51 ×16 cm flats containing a mixture of Norfolk sandy loam soil, sand, and peat (3:2:1 by volume). Isolations were made by triturating symptomatic leaf tissue in 0.05 M potassium phosphate buffer, pH 7.2, containing 0.1 M sodium diethyldithiocarbamate (1:10, w/v). Each plant extract was assayed on three *I. nil* 'Scarlet O'Hara' plants in the cotyledonary stage before the first true leaf was fully emerged.

In a separate experiment, isolations were made with aphids and mechanically from plants collected in Johnston County. *Aphis gossypii* and *Myzus persicae* reared on *Cucumis melo* and *Brassica juncea*, respectively, were given 10–20 sec acquisition access on symptomatic sweet potato leaves and 2-hr inoculation access on *I. nil* cotyledons.

Symptom development in *I. nil* from mechanical or aphid inoculation was initially observed for 14–21 days. Plants from each location were grouped by symptom type, and additional transfers were made to determine the stability of symptom types. An isolate representative of each group was passed through a dilution series followed by serial aphid transfer (11) to reduce the probability of a mixed infection. Isolates were maintained in individual screen cages in a screened greenhouse.

Host range. Isolates were tested on *I. nil, I. setosa, I. lacunosa, I. wrightii, I. purpurea, I. hederaceae, I. tricolor, I. batatas* 'Jersey' and 'Jewel,' *Chenopodium amaranticolor, C. quinoa, Cucumis sativus* 'National Pickling,' *Nicotiana tabacum* 'Burley 21,' *Phaseolus vulgaris* 'Kentucky Wonder,' and *Vigna unguiculata* 'Early Ramshorn.'

Inoculations were made to *N. tabacum* plants in the three to five leaf stage and to *Chenopodium* plants with fully expanded leaves; cotyledons of all other species were inoculated before emergence of the primary leaf. Mature and juvenile leaves from all plants were each indexed on five *I. nil* seedlings approximately 21 days after inoculation. Biological properties were determined using systemically infected *I. nil* leaves as a source and assayed on 5–10 *I. nil* seedlings.

Particle morphology was determined by using serologic specific electron microscopy (7) with anti-FMV coated grids. Electron micrographs were taken with a Siemens Elmiskop 1A electron microscope, and magnification was calibrated with a carbon replica grating at 2,160 lines per millimeter (E. F. Fullam, Inc., Schenectady, NY 12301). Serologic relationships were determined with leaf tissue extracts. Extracts for microprecipitin tests (3) were triturated in nine

volumes of 0.05 M phosphate buffer, pH 7.2, squeezed through two layers of cheesecloth and centrifuged (8,000 rpm, 10 min SS-34 rotor) to remove remaining debris. Antisera dilutions were made in Tris-NaCl buffer (0.05 M Tris, pH 7.2, containing 0.9% NaCl). Gel diffusion tests were conducted as previously described (9).

RESULTS

Isolation. Isolation attempts from FMV-infected Jewel and Porto Rico plants were only successful from symptomatic leaves, classes 3 and 4 (Table 1). Virus was not recovered from younger symptomless leaves. Other attempts to isolate virus from symptomless leaves on FMV-infected sweet potato plants using other buffers and stabilizing agents also failed (J. W. Moyer, *unpublished*).

Survey. A virus was isolated from 91 of the 175 sweet potato plants assayed from the 18 fields. Two distinct symptom types emerged following serial dilutions on *I. nil* indicator plants. The two types were subsequently designated FMV and RC-FMV based on disease reaction in Jersey sweet potato (Table 2). The FMV and RC-FMV type isolates were obtained at 14 and 17 locations, respectively, of the 18 locations surveyed. Both FMV and RC-FMV isolates were obtained from the second survey in Johnston County where mechanical and aphid transmission was used for isolation from sweet potato. Both isolates were obtained by both methods, but aphid transmission was less efficient (8 of 57) than mechanical transmission (41 of 57). Precautions, described above, were taken to reduce the probability of mixed infections. An isolate representative of each symptom type was then used for comparative studies.

Symptoms in FMV-infected *I. nil* plants were characteristic of those previously described (11). Symptoms of RC-FMV were expressed 7–14 days after inoculation. Symptoms in the primary leaf appear as veinal chlorosis, with the entire leaf becoming mildly chlorotic. The next one to three leaves exhibit light green vein banding of the major veins only, giving a water-soaked appearance; some leaves become slightly twisted or distorted. Subsequent growth was symptomless. Stunting was more severe with FMV than with RC-FMV.

The two isolates were compared in a host range of selected *Ipomoea* species representing frequently cited virus indicator plants, potential weed hosts, two sweet potato cultivars, and other useful virus indicators (Table 2). Veinal chlorosis or clearing followed by chlorotic vein banding of the primary veins was a characteristic symptom of both isolates on most *Ipomoea* spp. Borders of the vein banding were occasionally smooth but were most often irregular, resembling the margins of a feather. Infection of two other *Ipomoea* spp. gave distinct symptoms.

I. purpurea, although susceptible to both strains, remained symptomless when infected with FMV; however, RC-FMV induced vein clearing and banding similar to foliar symptoms exhibited by other *Ipomoea* spp. Both strains induced severe wilting and stem necrosis in *I. tricolor*. Foliar symptoms in the two sweet potato cultivars consisted of vein banding and chlorotic spotting. RC-FMV induced external, necrotic lesions characteristic of the russet crack disease (5,6). The host range was confirmed with an isolate of RC-FMV passed through three single lesion transfers on *C. amaranticolor*.

Six additional species from four families were inoculated. Infection of *C. amaranticolor* and *C. quinoa* by RC-FMV resulted in chlorotic local lesions. Indexing of the four symptomless species inoculated with RC-FMV and the six species inoculated with FMV indicated that infection had not occurred. Although the symptoms of the two isolates were very similar for most of the *Ipomoea* spp., sufficient differences existed to construct a differential host range. The common strain of FMV can be differentiated from RC-FMV by symptoms in *I. nil*, symptomless infection of *I. purpurea*, absence of local lesions in *C. amaranticolor* and *C. quinoa*, and the absence of root necrosis in Jersey sweet potato (Table 2).

A second survey was done in 1979 to confirm the representative nature of the isolates obtained in 1977. Symptomatic Jewel plants collected from nine fields in Columbus, Johnston, Nash, and Sampson counties were assayed mechanically on *I. nil*; a single *I. nil* plant with symptoms similar to FMV or RC-FMV was selected from each location, and the isolate was passed through at least two dilution series and grouped as FMV or RC-FMV based on symptoms in *I. nil*. A total of seven FMV and nine RC-FMV isolates were assayed on *C. quinoa*. All RC-FMV isolates produced chlorotic lesions on *C. quinoa*; no lesions were observed with the FMV isolates. All isolates reacted with FMV antisera in microprecipitin tests.

Other measured biological and physical properties were the same for both virus strains. Stability tests of both strains in plant extracts were the same: dilution end point, 10^{-3} to 10^{-4}; thermal inactivation

Table 1. Recovery of sweet potato feathery mottle virus from leaf tissues at various stages of development

	Leaf age classes[a]			
	Symptomless		Symptomatic	
Cultivar	1	2	3	4
Jewel	0/20[b]	0/20	19/20	18/20
Porto Rico	0/20	0/20	19/20	17/20

[a] Class: 1 = the two youngest leaves, 2 = oldest symptomless leaves, 3 = mature symptomatic leaves, 4 = young symptomatic leaves produced from lateral buds at the base of the vine.
[b] Numbers of infected/inoculated *I. nil* plants.

Table 2. Symptoms induced by FMV and RC-FMV sweet potato virus strains

	Diagnostic symptoms[a]	
Indicator species	FMV	RC-FMV
Ipomoea nil		
'Scarlet O'Hara'	S:VC, Cr, CS, R	S:VC, WSVB, R
setosa	S:VC, CVB, R	S:VC, CVB, R
tricolor 'Heavenly Blue'	S:W, SN, CVB	S:W, SN, CVB
purpurea	Symptomless	S:VC, CVB
lacunosa	S:VC, CVB	S:VC, CVB
wrightii	S:VC, CVB	S:VC, CVB
hederaceae	S:VC, CVB	S:VC, CVB
batatas 'Jersey'	S:CVB, CS	S:CVB, CS, RN
batatas 'Jewel'	S:CVB, CS	S:CVB, CS
Chenopodium		
amaranticolor	NI	L:CS
quinoa	NI	L:CS
Cucumis sativus 'National Pickling'	NI	NI
Nicotiana tabacum 'Burley 21'	NI	NI
Phaseolus vulgaris 'Kentucky Wonder'	NI	NI
Vigna unguiculata 'Early Ramshorn'	NI	NI

[a] Letters left of colon: S = systemic, L = local. Letters right of colon: CR = crinkling, CS = chlorotic spots, CVB = chlorotic vein banding, NI = not infected, R = recovery, RN = root necrosis, SN = stem necrosis, VC = vein clearing and subsequent chlorosis, W = wilting, WSVB = water-soaked vein banding.

point, 60–65 C; and longevity in vitro, less than 24 hr (25 C). Neither *A. gossypii* nor *M. persicae* transmitted the isolates persistently. Electron micrographs of plant extracts of both strains revealed flexuous rods 810–860 nm long.

The two strains are serologically related as determined by microprecipitin tests. Extracts from healthy plants and from RC-FMV and FMV infected *I. nil* plants incubated with anti-FMV serum gave dilution (reciprocals) end points of 0, 256, and 1024, respectively. Reactions in gel diffusion tests were inconsistent because of problems usually associated with rod-shaped viruses. Precipitin bands were diffuse and the antigen, particularly RC-FMV, did not always move into the gel. Further serologic studies await the development of purification procedures for RC-FMV. The purification protocol for FMV (11) was inadequate for RC-FMV because of severe aggregation of the particles.

DISCUSSION

Sap transmission from symptomatic sweet potato leaf tissue to *I. nil* seedlings is an efficient way to isolate known FMV strains. Studies attempting to free cultivars from virus through heat therapy or meristem-tip culture have showed that viruses occur in very young as well as old tissue (2,10). In our experience, however, isolation by sap transmission was successful only from symptomatic tissue. Grafting to *I. setosa* remains the method of choice for indexing symptomless plants.

Two FMV strains were distributed throughout the sweet potato growing region of North Carolina. One strain, which causes the russet crack disease of Jersey sweet potato, was designated RC-FMV because it was similar to the russet crack strain previously described (5). In that study, similar host range, particle morphology, and evidence for cross-protection strongly suggested that the russet crack agent is a strain of FMV; however, differential hosts to adequately separate the two strains were not identified (5).

We found *I. nil, I. purpurea,* and *C. amaranticolor* to be useful differential hosts for the two strains. The distinct symptom types produced in *I. nil* and *I. purpurea* (Table 2) provide a preliminary separation of strains; the absence of local lesion production by FMV and local lesion transfer of RC-FMV were used as criteria for obtaining pure cultures.

Symptoms produced on the expanded *Ipomoea* host range by the two strains and preliminary serologic studies further support previous findings that the russet crack virus is a strain of FMV. Although preliminary double-diffusion tests indicated that the two strains were not related, microprecipitin tests clearly indicated that the strains were related. Further comparison of the two strains will require the development of purification procedures to overcome the severe aggregation of RC-FMV.

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LITERATURE CITED

1. ALCONERO, R. 1973. Mechanical transmission of viruses from sweet potato, Phytopathology 63:377-380.
2. ALCONERO, R., A. G. SANTIAGO, F. MORALES, and F. RODRIGUEZ. 1975. Meristem tip culture and virus indexing of sweet potatoes. Phytopathology 65:769-773.
3. BALL, E. M. 1974. Serological Tests for the Identification of Plant Viruses. American Phytopathological Society, St. Paul, MN. 31 pp.
4. CALI, B. B., and J. W. MOYER. 1979. Differential properties of three sweet potato virus strains. (Abstr.) Phytopathology 69:1023.
5. CAMPBELL, R. N., D. H. HALL, and N. M. MIELINIS. 1974. Etiology of sweet potato russet crack disease. Phytopathology 64:210-218.
6. DAINES, R. H., and W. J. MARTIN. 1964. Russet Crack, a new virus disease of sweetpotatoes. Plant Dis. Rep. 48:149-151.
7. DERRICK, K. S. 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 55:652-653.
8. DOOLITTLE, S. P., and L. L. HARTER. 1945. A graft transmissible virus of sweet potato. Phytopathology 35:695-704.
9. GOODING, G. V., JR., and W. W. BING. 1970. Serological identification of potato virus Y and tobacco etch virus using immunodiffusion plates containing sodium dodecyl sulfate. (Abstr.) Phytopathology 69:1293.
10. HOLLINGS, M., O. M. STONE, and K. R. BOCK. 1976. Purification and properties of sweet potato mild mottle, a white-fly borne virus from sweet potato (*Ipomoea batatas*) in East Africa. Ann. Appl. Biol. 82:511-528.
11. MOYER, J. W., and G. G. KENNEDY. 1978. Purification and properties of sweet potato feathery mottle virus. Phytopathology 68:998-1004.
12. NUSBAUM, C. J. 1945. A preliminary report on internal cork, a probable virus disease of sweet potato. Plant Dis. Rep. 29:677-678.