A COMPARISON OF PUMPKIN MOSAIC VIRUS AND ISOLATES OF SQUASH MOSAIC VIRUS

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ABSTRACT

Pumpkin mosaic virus (PMV) (23), a squash mosaic virus (SMV) type culture (25), and six isolates of SMV from Kansas were compared. PMV has nearly the same host range as SMV and like the serological Group II of SMV (16) does not infect watermelon. Three genera in the *Leguminosae* were susceptible to PMV only and two genera in the *Umbelliferae* were susceptible to SMV isolates only. Physical properties were similar. Dilution end point was 10^{-4} for PMV and 10^{-4} to 10^{-5} for SMV; thermal inactivation point was 80 for PMV and 70-75 C for SMV, and longevity *in vitro* was 21 days for PMV and 20-24 days for SMV at 20 C. Using plant height and dry weight as criteria, the virulence of the six Kansas SMV isolates plus PMV (23) and SMV (25) were compared in three squash cultivars. All eight significantly reduced plant height and dry matter content as compared to the uninoculated controls. Under greenhouse conditions, the most severe isolates of SMV caused an average reduction in plant height of 36% for the three cultivars, the mildest isolate a reduction of 23%. Buttercup squash plant height reduction was 21% for the mild isolate, 28% for an intermediate, and 52% for a severe isolate. PMV (23) caused a 52% height reduction in Buttercup, whereas SMV (25) reduced it by only 25%. Dry weight results were somewhat inconsistent but isolate G of SMV was consistently and significantly more severe than the others, while isolate F was typically milder. Butternut squash showed more tolerance to both PMV and SMV than the other cultivars. The six Kansas isolates were separated into 3 tentative SMV strains, mild, intermediate and severe, based upon virulence (plant height and dry weight) on Early Prolific Straightneck Squash. This work plus serology and electron microscopy studies published earlier by El-Hadidi et al. (4) indicates a close relationship between PMV (23) and all SMV isolates including an SMV type culture (25). PMV appears to be a strain of SMV in the serological Group II (16) differing slightly in host range.

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Cucurbit viruses are generally distributed over the United States (1, 5, 10, 12, 14, 24) and frequently infected plants contain two or more viruses (6, 22).

The most prevalent viruses infecting cucurbits in Kansas were strains of squash mosaic virus (SMV) as reported by Salama (19) and Salama and Sill (20). SMV accounted for 46% of all cucurbit viruses in Kansas surveys during 4 years. A new virus tentatively named pumpkin mosaic virus (PMV) by Stoner (23) in South Dakota was compared in this study with Kansas isolates and a known SMV strain (Webb) (25) to ascertain its relationship with SMV. Symptomatology, host range, physical properties and disease severity were the criteria used to establish possible relationships. Comparative serological and electron microscopy data appeared in an earlier report (4).

MATERIALS AND METHODS

Physical Properties. In all experiments the virus-containing plant extract was obtained by macerating infected leaves of Early Prolific Straightneck squash in mortar and pestle. The pulp of ground leaves was collected in cheese cloth and the extract was squeezed through by hand. The collected sap was diluted with distilled water to a ratio of 1 to 10. Separate extracts from each virus isolate were used to determine dilution end points, thermal inactivation points, and longevity *in vitro*. For dilution end point experiments, serial 10-fold dilutions in sterile distilled water were prepared in sterile test tubes. Mechanical inoculations of each dilution were made immediately to cotyledons of 10 Early Prolific Straightneck squash plants. These plants were observed for appearance of symptoms and finally were scored positive or negative four weeks after inoculation.

In thermal inactivation experiments, the diluted (1-10) plant extracts were placed in capillary tubes (25 cm x 4 mm O. D.) which were sealed in a gas flame. The sealed end was inserted through a cork until the surface of the virus extract was about 1 cm below the bottom of the cork. During the heat treatment the capillaries were floated in a constant temperature water bath with the surface of the extracts approximately 4 cm below the water surface. Temperatures used were 50, 55, 60, 65, 70, 75, 80, and 85 C. After ten minutes the capillaries were removed and immersed in ice water. Small fluctuations in water temperature, approximately + or −1 C, were present during thermal treatments and increased slightly with an increase in temperature. All inoculations were made within one day of preparation of the inocula. Ten young squash plants were inoculated on the cotyledons with each suspension. Observations and scoring were the same as those used in dilution end point experiments.

Aging *in vitro* experiments were conducted with 1:10 dilution of plant extracts from each isolate. Extracts were stored at room temperature, in the greenhouse, and in a growth chamber at 20 C. Inoculations to 10 Early Prolific Straightneck squash plants were made the day the virus containing extract was prepared and every second day thereafter for a maximum of 24 days. Final readings were made four weeks after inoculation.

Quantitative Virulence Studies of SMV and PMV on Three Squash Cultivars. Both field and greenhouse experiments designed to obtain quantitative data on the virulence of SMV isolates and PMV were conducted using 3 summer squash cultivars. Inoculum was sap extracted from infected Early Prolific Straightneck squash leaf tissue and diluted 1:10 with sterile distilled water. All plants were inoculated at the true two-leaf stage by the carborundum

Six Kansas isolates of SMV (E, F, G, J, P, and R) were used. Each of the six isolates plus PMV (23) and an SMV type culture (25) was inoculated to 15 seedlings each of Early Prolific Straightneck, Butternut, and Buttercup squash. Plants were observed for severity of infection. After three months growth, the average height of each variety was measured. The plants were then cut and dried rubbing method. Healthy controls were given no treatment. in paper sacks for 24 hours at 90 C and dry weights were recorded. The experiments were repeated three times.

Winter greenhouse temperatures were 21 during the day and 15 C at night. Summer daytime temperatures averaged 30 with 21 C at night. To reduce summer temperatures in the greenhouse, shading compound was applied and both evaporative cooler and mist spraying systems were used. Hyponex fertilizer was used as needed. The greenhouse was fumigated once per week with Plantfume 103; Malathion also was used as needed in both greenhouse and field.

Symptomatology & Host Range. A selection of plants from *nine* families including 26 genera and 30 species was inoculated with each Kansas isolate as well as SMV (Webb) (25) and PMV (23) to determine the host range, and to study symptomatology in different hosts. The mechanical inoculation techniques used have been described by other investigators (11, 12, 21, 24, 25).

RESULTS

SMV has a narrow host range. All SMV isolates and PMV developed symptoms on all tested members of the *Cucurbitaceae* except watermelon. Chervil and Coriander in the *Umbelliferae* were infected by all SMV isolates but not by PMV. PMV infected the Garden pea, var. Little Marvel, Fenugreek and Lentil in the

TABLE 1

Reaction of Various Plants to Inoculation With Isolates of Squash Mosaic and Pumpkin Mosaic Viruses

Host Plant Scientific Name	Common Name	Reaction SMV	Reaction PMV
Solanaceae			
Nicotiana tabacum, L.	Tobacco	−	−
N. glutinosa, L.		−	−
Datura stramonium L.	Jimson-weed	−	−
Lycopersicon esculentum, Mill.	Tomato var. Marglobe	−	−
Capsicum annum L.	Pepper var. California Wonder	−	−
Chenopodiaceae			
Beta vulgaris L.	Sugar beet var. Early Wonder	−	−
Chenopodium amaranticolor Coste & Regn.		−	−
Compositae			
Zinnia elegans Jacq.	Zinnia var. Lilliput	−	−
Leguminoseae			
Soja max, Piper	Soybean	−	−
Phaseolus limensis Macf.	Lima Bean	−	−
Vigna sinensis (L.) Savi	Cowpea var. Black	−	−
Pisum sativum L.	Garden Pea var. Little Marvel	−	+
Trigonella Foenum-Graecum L.	Fenugreek	−	+
Lens culinaris, Medic.	Lentil	−	+
Umbelliferae			
Chaerophyllum bulbosum, L.	Chervil	+	−
Coriandrum sativum, L.	Coriander	+	−
Cucurbitaceae			
Cucurbita Pepo, L.	Field Pumpkin	+	+
C. Pepo Var. *Melopepo*, Alef.	Squash var. Early Prolific Straightneck	+	+
Cucumis sativus, L.	Cucumber var. Palomar	+	+
Citrullus vulgaris, Schrad.	Watermelon var. Charleston Gray	−	−

\+ positive for systemic infection
− negative reaction

Leguminoseae (Table 1), but SMV isolates did not. None of the inoculated plants from the *Compositae*, *Chenopodiaceae*, and *Solanaceae*, were susceptible to either PMV or SMV isolates (Table 1). This was also true for members of the *Amaranthaceae*, *Malvaceae*, and *Liliaceae* tested earlier.

Physical Properties. Using Early Prolific Straightneck squash both as a source and test plant, the dilution end point, thermal inactivation point and aging *in vitro* were determined. Stoner's PMV (23) and Webb's SMV (25) were compared with 3 (mild, intermediate and severe) Kansas SMV isolates.

Isolates E, G and SMV (25) induced occasional infections when diluted to 10^{-5} but not at 10^{-6} while the dilution end point of F and PMV was 10^{-5}. Infectivity percentages dropped markedly for all SMV isolates and PMV at 10^{-4} (Table 2).

TABLE 2

Dilution End Point of Three Isolates of SMV as Compared to a Type Strain and PMV

Dilution	Isolates of SMV[a] E	Isolates of SMV[a] F	Isolates of SMV[a] G	PMV (Stoner)	SMV (Webb)
0	30/30[b]	30/30	30/30	30/30	30/30
10^{-1}	30/30	30/30	30/30	28/30	30/30
10^{-2}	30/30	27/30		29/30	27/30
10^{-3}	28/30	26/30	15/30	20/30	18/30
10^{-4}	15/30	12/30	11/30	14/30	17/30
10^{-5}	1/30	0/30	5/30	0/30	4/30
10^{-6}	0/30	0/30	0/30	0/30	0/30

[a]Results are total of three trials in Early Prolific Straightneck squash.
[b]Numerator is number of plants infected; denominator is number inoculated.

Thermal inactivation of all SMV isolates occurred between 70-80 C during a 10 minute exposure. The thermal inactivation point was between 70 and 75 C for isolates F and G. Isolate E, PMV and SMV (25) had some infectivity remaining after heating to 75 while one plant of 30 inoculated in 3 trials with PMV heated to 80 C was infected (Table 3).

TABLE 3

Thermal Inactivation of Three Isolates of SMV as Compared to a Type Strain and PMV

Temperature (°C)	Isolates of SMV[a] E	F	G	PMV (Stoner)	SMV (Webb)
Unheated	30/30[b]	30/30	30/30	30/30	30/30
50	28/30	29/30	27/30	28/30	28/30
55	25/30	24/30	28/30	26/30	24/30
60	21/30	18/30	20/30	26/30	20/30
65	13/30	7/30	9/30	15/30	11/30
70	5/30	3/30	5/30	8/30	6/30
75	2/30	0/30	0/30	3/30	2/30
80	0/30	0/30	0/30	1/30	0/30
85	0/30	0/30	0/30	0/30	0/30

[a]Results are total of three experiments in Early Prolific Straightneck squash.
[b]Numerator is number of plants infected; denominator is number inoculated.

When juice was stored at 20 C, infection occurred up to 24 days with both E and G isolates. Isolate F was infectious up to 20 days but not 21 days. Aging *in vitro* for SMV (25) was 22 days and for PMV (23) 21 days (Table 4).

Quantitative Virulence Studies (Plant Height and Dry Weight) of SMV Isolates and PMV on Three Squash Cultivars. The comparative virulence of the isolates of SMV and PMV was investigated in both greenhouse and field plot experiments. Field data are not recorded here, but supported and confirmed the greenhouse data. The greenhouse data are reported in Table 5.

The variety of symptoms in Early Prolific Straightneck squash plants suggested the existence of different strains of SMV which vary greatly in their virulence. In quantitative studies (plant height and dry weight) on this host the 6 SMV isolates were classified on the basis of disease severity as 3 strains, mild (F), intermediate (E, P, R), and severe (G, J), (Table 5).

The average mean plant height for all virus isolates was 13.10, 24.18, and 60.93 inches for Early Prolific Straightneck, Butternut and Buttercup squash, respectively, whereas the healthy controls

TABLE 4

Aging **in vitro** at 20 C of Three Isolates of SMV as Compared to a Type Strain and PMV

Days	Isolates of SMV[a] E	F	G	PMV (Stoner)	SMV (Webb)
0	10/10[b]	10/10	10/10	10/10	10/10
2	10/10	10/10	8/10	10/10	10/10
4	8/10	9/10	10/10	9/10	7/10
6	10/10	8/10	9/10	10/10	5/10
8	6/10	10/10	9/10	7/10	8/10
10	8/10	8/10	7/10	9/10	6/10
12	7/10	4/10	5/10	6/10	5/10
14	6/10	5/10	6/10	5/10	4/10
16	4/10	5/10	4/10	2/10	2/10
18	5/10	3/10	2/10	3/10	3/10
20	3/10	1/10	3/10	2/10	1/10
21	2/10	0/10	1/10	2/10	2/10
22	2/10	0/10	2/10	0/10	1/10
23	1/10	0/10	0/10	0/10	0/10
24	1/10	0/10	1/10	0/10	0/10
25	0/10	0/10	0/10	0/10	0/10
26	0/10	0/10	0/10	0/10	0/10

[a]Test host was Early Prolific Straightneck squash.
[b]Numerator is number of plants infected; denominator is number inoculated.

of the same cultivars were 16.40, 31.60, and 96.33 inches respectively (Table 5). On all three squash cultivars, Kansas isolates G and J were generally more severe and F was typically the mildest. Isolate G was the most virulent in terms of reduction of plant height but not significantly different from PMV which was also very virulent. Isolate J was somewhat less severe than G and there was a significant difference between J, PMV, and E. Plants inoculated with isolate E were significantly taller than with G. Those inoculated

TABLE 5

Effect of SMV Isolates and PMV on Plant Height (Inches) and Dry Weight (gm.) of Three Squash Cultivars in the Greenhouse

Isolates & Viruses Tested	Early Prolific Straightneck		Butternut		Buttercup	
	Height (in.)	Dry wt. (gm.)	Height (in.)	Dry wt. (gm.)	Height (in.)	Dry wt. (gm.)
E[a]	14.00	5.00	23.26	2.86	64.73	8.53
F	15.00	4.86	24.46	3.66	76.06	7.20
G	8.66	2.06	20.13	2.33	36.93	3.73
J	11.40	3.00	25.53	2.86	56.26	5.73
P	13.66	3.93	23.06	3.00	71.46	7.13
R	14.60	5.66	26.13	3.86	63.80	7.53
SMV (Webb)	15.53	3.33	29.20	2.86	72.33	8.26
PMV (Stoner)	12.00	3.00	21.73	2.80	45.93	4.60
Mean	13.10	3.85	24.18	3.02	60.93	6.58
Control	16.40	9.66	31.60	4.93	96.33	18.00

[a]Approximate virulence based upon all tests F = mild; E, P and R = intermediate; G and J = severe. PMV (Stoner) was quite severe whereas SMV (Webb) was quite mild.

[b]Each figure is an average of 45 plants in three successive experiments.

L.S.D. for plant height = 12.90 (5% level)

L.S.D. for dry weight = 3.55 (5% level)

with isolate R, with a mean 34.84 inch plant height, were significantly taller than with G and PMV. Among isolates J, E, R, P, and F there were no significant differences in the means; however, plants inoculated with F, P, and R were significantly taller than those with J, PMV, and G. SMV (25) inoculated plants were significantly taller than those inoculated with G, PMV, and J. There was no significance between SMV (25) and E, R, P, and F isolates. All SMV isolates studied, and PMV produced plants significantly shorter than the uninoculated controls.

The results (Table 5) of the analysis of variance of dry weights of infected plants grown in the greenhouse showed a significant interaction between cultivars and isolates. Isolate R was the least virulent in Early Prolific Straightneck squash, yet it still produced a mean dry weight that was significantly lower than that of the uninoculated control. The most severe isolates again were G and J. Infection with all isolates produced significantly less dry matter than the uninoculated controls.

In Buttercup squash the reaction of isolate E was least virulent, although the dry weight was significantly lower than the uninoculated control. Isolate G was again the most virulent followed by PMV and J. All isolates tested on Buttercup reduced dry weight significantly from that of the uninoculated controls (Table 5).

By contrast, in Butternut squash all isolates did not produce a significant reduction in dry matter when compared with uninoculated controls. Isolate G was the most virulent followed by J, PMV, E and P. Isolate F and R reacted mildly on Butternut compared to the others. Butternut was much more tolerant to all of the isolates tested than the other 2 cultivars.

DISCUSSION

It is possible to establish virus relationships on the basis of symptoms in susceptible hosts, host range, physical properties, serology, and cross-protection. These tests when carefully performed are generally regarded as accurate for identifying plant viruses (2, 18). In some cases there are no known methods for differentiation other than symptomatology and host range (18). We feel these 2 methods may be suitable for the identification of certain strains of SMV.

Diagnosis of strains of SMV based on differences in reactions produced on only one squash cultivar, such as the very susceptible Early Prolific Straightneck, has definite limitations. A set of suitable indicator plants to act as differential hosts for each strain would be more reliable if they could be found. However, until now the host plants tested as possible strain diagnostic hosts have not shown much promise except for the separation of the watermelon strain (15).

The length of the incubation period of the SMV isolates and PMV in this study did not vary much in any host and could not be used as a basis for diagnosis. Squash varieties tested were generally 100% infected; so differences in percent of infection could not be utilized. Leaf symptoms and resistance characteristics of the different hosts and cultivars used appeared to be the most promising basis for SMV strain differentiation.

The SMV isolates from Kansas were similar in host range and symptomatology to the SMV described by Middleton (13) and Freitag (5). All isolates in this study compared favorably in host range and physical properties with those of the squash mosaic group, but not with the watermelon virus in the study of Lindberg et al., (10).

Virulence was the outstanding variable feature of the SMV isolates tested. Differences in virulence is common in strains of other plant viruses also (17).

Based upon symptom severity on only one squash cultivar, Early Prolific Straighneck, the six Kansas isolates were separated into 3 strains, one mild, one intermediate, (3 isolates), and 1 severe (2 isolates).

Differences in severity and host range have been reported for other isolates of SMV. Lindberg et al. (10) isolated "severe squash mosaic" and "mild squash mosaic" from wild cucumber in Wisconsin. Nelson et al. (15) found that the Arizona strain of SMV caused systemic infection in watermelon whereas the common strain did not. Stoner's PMV (23), in like manner, apparently differs only in host range from common SMV. It infects three legumes (Pea, Lentil and Fenugreek) (Table 1), not infected by the other SMV isolates. PMV does not infect Chervil or Coriander (Table 1) whereas the other tested isolates of SMV do. Our serology and electron microscopy studies (4) compared favorably with those of Lastra and Munz (8) and also indicated a close relationship between PMV and SMV isolates. Hence, we consider that Stoner's PMV (23) is not a distinct virus but a strain of SMV differing only slightly in host range and not in serology.

In conclusion, it appears that there are several types of strain differences in SMV. The first would be the host range differences characteristic of the watermelon strain of Nelson et al. (15), the Y-48 strain of Demski (3), and Stoner's PMV (23). The second, as described in this study, would be characterized by consistent and significant differences in virulence in a suitable host plant. Third, some common strains tested and the Y-48 strain can be distinguished by cross protection studies on a local lesion host as described by Demski (3) and by variations in cross protection (reciprocal and non-reciprocal) as described by Lima and Nelson (9). Fourth, there are the serotype differences between the squash (including pumpkin) and watermelon strains, reported by Knuhtsen and Nelson (7) and Nelson and Knuhtsen (16). In an earlier report, no serological differences between PMV and various other SMV isolates were found (4), but these probably were all members of the serological Group II strains studied by Nelson and Knuhtsen (16) which do not infect watermelon. None of the strains described can be distinguished as yet by physical properties, electron microscopy, or electrophoretic mobility (4, 7).

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