Comparison of Tobacco Vein Mottling and Pepper Veinal Mottle Viruses

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ABSTRACT

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Cross-inoculation tests with tobacco and peppers showed that tobacco vein mottling virus (TVMV) infected tobacco but not peppers, whereas the type strain and three other strains of pepper veinal mottle virus (PVMV) infected peppers but caused limited, if any, systemic infection of tobacco. Serological tests readily differentiated TVMV and PVMV. One PVMV isolate (NTV) resembled TVMV in that it infected tobacco but not peppers. Serological tests showed the NTV isolate to be closely related to PVMV and only distantly related to TVMV. A newly introduced TVMV-resistant tobacco cultivar, Tennessee 86, varied in its response to the PVMV strains.

Additional keywords: aphid transmission, potyvirus resistance

Tobacco vein mottling virus (TVMV) is widely distributed throughout Kentucky and other southeastern states. This virus has become established in perennial solanaceous weeds and causes major losses in yield and quality of tobacco (*Nicotiana tabacum* L.), particularly burley types (5,6,10–12). Pepper veinal mottle virus (PVMV), first reported in Ghana (2), infects a number of solanaceous hosts, the most important of which are peppers, *Capsicum annuum* L. and *C. frutescens* L., in which it causes severe yield losses (4). PVMV is not known to occur in the United States (3). The type strain of PVMV does not infect tobacco systemically (3), but there are other isolates of the virus, from various regions in West Africa, that do (4). One isolate in particular, the Nigerian tobacco virus strain of PVMV (PVMV-NTV), produces symptoms in tobacco identical to those produced by TVMV (7). Electron microscope serology tests indicated that TVMV, PVMV, and PVMV-NTV are related (7).

Kentucky is the leading U.S. producer of both burley tobacco and peppers for processing, and these crops are often grown in close proximity. The studies reported here were undertaken to compare directly the response of tobacco and pepper cultivars to these viruses. The potential susceptibility to PVMV of Tennessee 86 (TN 86), a newly released burley cultivar resistant to TVMV, and the ability of peppers to serve as symptomless hosts of TVMV were of particular interest. The ability to distinguish TVMV and PVMV serologically was also determined.

MATERIALS AND METHODS

The isolate of TVMV was a subculture of the Clark County Ky isolate (12). Isolates of the PVMV type strain and strains PVMV-WA 23, PVMV-WA 31, and PVMV-WA 50, described previously (4), were provided by A. A. Brunt, Glasshouse Crops Research Institute,

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Littlehampton, Sussex, England. An isolate (A-T-M) of strain PVMV-NTV was provided by I. A. Roberts and A. F. Murant, Scottish Crop Research Institute, Invergowrie, Dundee, Scotland. All PVMV isolates were imported under USDA-APHIS permit. Infected plants were grown under conditions that precluded the introduction or escape of potential aphid vectors, and all plants used in the experiments described in this report were killed by steaming prior to being discarded. *N. benthamiana* Domin was the propagation host and the source of all viruses for mechanical inoculation. Aphid transmission tests were done with *Myzus persicae* (Sulzer) under strict confinement, by previously described procedures (9).

The plants used in the host comparison study were two cultivars of tobacco, Kentucky 14 (KY 14) (susceptible to TVMV) and TN 86 (resistant to it), and two species of peppers, *C. annuum* 'California Wonder' and *C. frutescens* 'Tabasco.' Tomato (*Lycopersicon esculentum* Mill.) was included, since it showed a differential response to PVMV isolates in a previous study (4); the cultivar Rutgers was used in the tests reported here. The presence of virus in symptomless leaves was detected by mechanical inoculation of *N. benthamiana* with leaf extracts. Both inoculated and potentially infected uninoculated leaves were assayed if no symptoms were produced in the test cultivars. If symptoms appeared on inoculated leaves with no evidence of systemic infection, leaves above the inoculated leaf were assayed 3 and 5 wk after inoculation. The type or presence of symptoms on inoculated leaves was not recorded if systemic symptoms appeared in the test cultivar.

Serological tests were performed with virus purified by method 1 of Mohgal and Francki (8). Virus concentrations were calculated spectrophotometrically, with the extinction coefficient $E(0.1\%/260\ nm) = 3$ (8). Antiserum to TVMV was prepared by injecting rabbits with the purified virus; antiserum to the type strain of PVMV was generously provided by A. A. Brunt. Indirect enzyme-linked immunosorbent assay (ELISA) was performed as described by Berger et al (1), using goat antirabbit alkaline phosphatase as the detection system. Tests were performed with duplicate samples, and a reaction was considered positive when the absorbance at 405 nm was at least 2 standard deviations above the mean background level. Ring precipitin tests were done as described by Walkey (13); purified virus at 20 μg/ml was layered over a series of antiserum dilutions made in glycerine (final concentration 25%, v/v) and 0.85% NaCl. The presence or absence of a precipitate was determined visually after 2 hr of incubation at room temperature.

RESULTS

Host susceptibility. Inoculation with TVMV resulted in symptom production only in KY 14. The virus could be recovered only from inoculated leaves of TN 86 and from both inoculated and systemically infected tomato. It could not be recovered from either pepper cultivar (Table 1).

The type strain and strains WA 23, WA 31, and WA 50 of PVMV all infected tomato and both cultivars of peppers sytemically; the symptoms produced varied somewhat with the isolate. The reaction of the tobacco cultivars to these isolates was more variable, but in no case was a severe systemic disease produced, nor was there a consistent differential response of the two tobacco cultivars. The type strain and strain WA 31 sometimes produced ring spots or chlorotic spots on systemically infected tobacco leaves, but these were extremely sporadic; most leaves were symptomless, and the spots, when present, covered less than 0.5% of the area of a leaf. Strains WA 23 and WA 50 could be recovered only from inoculated leaves (Table 1).

The NTV isolate of PVMV differed from the other PVMV isolates in that it did not produce symptoms in peppers and was not detected in inoculated or uninoculated leaves. It was the most severe of the isolates on tomato, in which it caused lethal necrosis. The two tobacco cultivars reacted differentially to this isolate, but the responses differed from those to TVMV. PVMV-NTV produced a more general mottle in KY 14 and occasional systemic symptoms in TN 86 (Table 1).

Aphid transmission. Transmission tests with aphids were done to determine the possible importance of infected symptomless tobacco leaves as sources of the natural spread of PVMV. Aphid

Table 1. Symptoms produced on greenhouse-grown tobacco, pepper, and tomato cultivars mechanically inoculated with tobacco vein mottling virus (TVMV) and strains of pepper veinal mottle virus (PVMV)

		Symptoms[b]				
		Tobacco[c]		Pepper		Tomato
Virus	Leaf tested[a]	KY 14	TN 86	California Wonder	Tabasco	Rutgers
TVMV	I	—	0+	0	0	0+
	S	VC, VM	0	0	0	0+
PVMV type strain	I	(CS, RS)	(NS)	—	—	(N)
	S	0+	(RS)	VM, D	M	SN
PVMV-WA 23	I	RS, CS	RS, CS	—	—	N
	S	0	0	VM	M	SN
PVMV-WA 31	I	0+	0+	—	—	N
	S	(CS)	(CS)	VM	M, Mo	N, D
PVMV-WA 50	I	0+	0+	—	—	0
	S	0	0	VC, VM	VC, VM	M, D
PVMV-NTV	I	VC	0+	0	0	N
	S	M, VM	(CS)	0	0	LN

[a] I = inoculated leaf; S = potentially systemically infected leaf.
[b] — = Not tested or not recorded; 0 = symptomless, virus not recovered; 0+ = virus recovered from symptomless leaves; CS = chlorotic spots; RS = ring spots; NS = necrotic spots; D = distortion; M = mottle; Mo = mosaic; N = necrosis; SN = severe necrosis; LN = lethal necrosis; VC = veinclearing; VM = vein mottling. Parentheses indicate that the symptom was very sporadic.
[c] KY 14 = Kentucky 14; TN 86 = Tennessee 86.

Table 2. Transmission of pepper veinal mottle virus (PVMV) and tobacco vein mottling virus (TVMV) from mechanically inoculated plants by *Myzus persicae*

Virus	Source plant[a]	Symptoms[b]	Transmission[c]
PVMV type strain	Tobacco (KY 14)	+	17/20
	Tobacco (KY 14)	–[d]	1/30
	Tobacco (TN 86)	+	19/20
	Tobacco (TN 86)	–[d]	0/20
	Pepper (California Wonder)	+	17/20
TVMV	Pepper (California Wonder)	–	0/60
	Tobacco (TN 86)	–	0/60
	Tobacco (KY 14)	+	20/20

[a] KY 14 = Kentucky 14; TN 86 = Tennessee 86.
[b] Uninoculated leaves were used as the virus source.
[c] Numerator, number of plants infected; denominator, number of test plants. The test plants were KY 14 tobacco for TVMV and California Wonder pepper for PVMV. Ten aphids were placed on each test plant. The data are totals from two or three experiments.
[d] A nonsymptomatic area of a symptomatic leaf was used for virus acquisition by aphids.

Table 3. Relatedness of tobacco vein mottling virus (TVMV) and two strains of pepper veinal mottle virus (PVMV) as determined by ring precipitin tests

Antigen	Antiserum[a]	
	TVMV	PVMV
TVMV	512	NR[b]
PVMV type strain	4	256
PVMV-NTV	16	128

[a] Reciprocal of the highest dilution at which a visible precipitate formed. The tests were performed in duplicate, with identical results.
[b] NR = no detectable reaction.

transmission tests were also done with TVMV-inoculated peppers. Although virus could not be recovered from such plants by mechanical inoculation, peppers are known to contain inhibitors of mechanical transmission; aphid transmission is not affected by such inhibitors.

The results (Table 2) show that PVMV could be readily aphid-transmitted from the symptomatic areas that occasionally developed on systemically infected tobacco leaves, but the transmission level was much lower from adjacent non-symptomatic tissues. No transmission of TVMV occurred from TN 86 tobacco or from pepper. A high level of transmission occurred from the controls (TVMV in KY 14 and PVMV in pepper).

Serology. Both ring precipitin tests (Table 3) and ELISA (Fig. 1) using purified viruses and antisera to TVMV and the PVMV type strain showed a close relationship between the PVMV type strain and PVMV-NTV; these viruses were only distantly related, serologically, to TVMV.

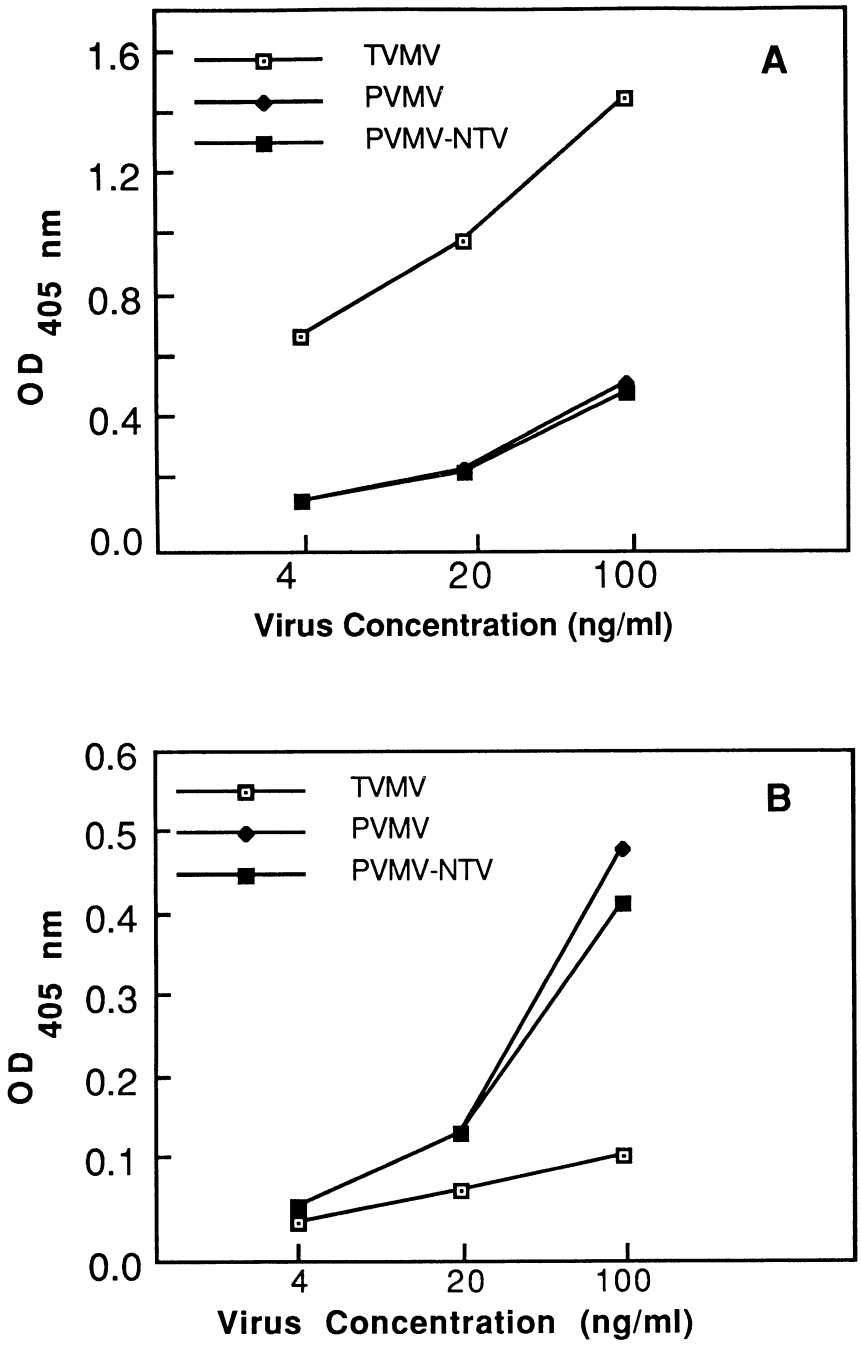

Fig. 1. Serological relatedness of tobacco vein mottling virus (TVMV) and two strains of pepper vein mottling virus (PVMV) as determined by indirect enzyme-linked immunosorbent assay with anti-TVMV immunoglobulin G (IgG) (**A**) and with anti-PVMV (type strain) IgG (**B**). The concentration of rabbit IgG was 1 μg/ml in both cases. Goat antirabbit conjugated alkaline phosphatase was used to quantitate the binding of the primary antibody. Background OD was less than 0.005 in all tests and is indistinguishable from the bottom line of the graph on these scales.

DISCUSSION

The results reported here show that TVMV can be readily distinguished from the type strain and the WA 23, WA 31, and WA 50 isolates of PVMV on the basis of the differential susceptibility of the hosts of economic importance in Kentucky, tobacco and peppers. Whereas a previous report indicated a relatively close serological relationship between TVMV and PVMV, as determined by electron microscope serology (7), the results reported here show that the two viruses can be readily differentiated by ring precipitin and ELISA tests. The reason for the apparent discrepancy may lie in differences in the methodology, but the data reported here support the view in which TVMV and PVMV are regarded as different potyviruses.

The relationship between TVMV and the NTV isolate of PVMV is less clear-cut. The NTV isolate resembled TVMV in its ability to infect KY 14 systemically and in its restricted spread in TN 86. The symptoms produced by NTV in KY 14 differed from those produced by TVMV, and the symptoms also differed from those described and depicted in tobacco by Ladipo and Roberts (7), which were virtually identical to those caused by TVMV. The NTV isolate used in this study also differed from the one described originally (7) in its inability to infect Tabasco and other peppers (and it is similar to TVMV in that respect).

On the basis of the differential reaction of the hosts of interest, tobacco and pepper, the NTV isolate used in this study would seem to be closer to TVMV than PVMV. However, the serological data show that NTV is virtually identical to PVMV and only distantly related to TVMV. This type of discrepancy between serological and host range data is common with potyviruses. From the standpoint of serology NTV is a strain or isolate of PVMV; from the standpoint of a practical plant pathologist in the United States, the introduction of the type strain of PVMV could pose a threat to peppers but not to tobacco, whereas the NTV strain of PVMV could pose a threat to tobacco but not to peppers.

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