Biological and Serological Characterization and Separation of Potyviruses that Infect Peppers

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ABSTRACT

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Potato virus Y (PVY), tobacco etch virus (TEV), an unidentified pepper virus from North Carolina (NC), and five isolates of pepper mottle virus (PeMV) from Arizona, California, New Mexico, and Florida were compared for host range, cross protection, and serology. The NC virus was related serologically to the PeMV strains. Otherwise, no close serological relationship existed between any of the viruses. The NC virus , however, did not share the most characteristic feature of the PeMV isolates: induction of necrotic local lesions on *Capsicum frutescens* 'Tabasco'. Cross protection tests between NC and PeMV did not indicate a relationship. Strains of PeMV showed marked and consistent variation in the extent of secondary necrosis on tabasco, severity on *Nicotiana tabacum* 'Xanthi' and on several pepper breeding lines, including fruit distortion (or lack of it) on *C. frutescens* 'Anaheim', and susceptibility of *Chenopodium amaranticolor*. Host reactions were found adequate for differentiating between these viruses, unless certain mixtures occurred. In such mixtures, PVY could be detected only by serology.

Viruses of the potyvirus group are widespread in pepper growing areas of the U. S. (4, 6, 7, 8, 9, 13, 14). For many years the two most commonly recognized members of this group have been potato virus Y (PVY) and tobacco etch virus (TEV). Recently a new virus belonging to the potyvirus group was found in Arizona (9) and Florida (11). The virus is serologically distinct from PVY and TEV and causes characteristic local lesions on, and subsequent systemic necrosis and premature death of, the chili pepper, *Capsicum frutescens* L. 'Tabasco'.

There is evidence for the occurrence of this new virus in years past in California and Florida where it was lumped with TEV or PVY strains. One earlier publication (4) reported the isolation of a group of TEV strains that produced local lesions on and death of tabasco pepper. More recently, several such isolates have been described as strains of PVY (8, 14).

The first recognized and serious outbreak of a disease caused by this new virus occurred in 1969 in pepper fields near Elfrida, Arizona, probably as the result of the use of transplants from California (9). The virus was initially assumed to be an isolate of either TEV or PVY, but serological reactions and the distinctive effect on tabasco pepper indicated otherwise (9, 11). Although the unique symptoms induced in tabasco pepper suggest that "tabasco necrosis virus" would be a logical name, there exists the obvious problem of potential confusion of this name with the common tobacco necrosis virus. We therefore concur with the use of the name pepper mottle virus (PeMV) (11).

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MATERIALS AND METHODS

Virus isolates.—Sources of PeMV isolates were (i) Elfrida, Arizona: one each from *Datura meteloides* (PeMV-AzD) and *Capsicum frutescens* L. 'New Mexico 6-4' (PeMV-AzP); (ii) Irvine Ranch, near Newport Beach, California: one from *C. frutescens* L. 'Anaheim' (PeMV-Cal); (iii) Hatch, New Mexico: one from 'New Mexico 6-4' (PeMV-NM); and (iv) Belle Glade, Florida: one from the bell pepper, *C. annuum* L. 'Early Calwonder' (PeMV-Fla). Other virus isolates included TEV (PV-69) from the American Type Culture Collection and PVY (NC 57), in addition to an unknown pepper virus (NC), from G. V. Gooding, Jr., North Carolina State University, Raleigh, NC 27607.

Hosts.—Plants tested included commercial pepper cultivars, pepper breeding lines, certain standard virus indicators, and native weeds which commonly occur in papper growing regions in Arizona.

Companies that provided seeds of commercial pepper cultivars included Asgrow, Ferry-Morse, Niagara, Northrup-King, and Petoseed. Such cultivars included California Wonder, Chinese Giant, Early Calwonder, Early Pimento, Fordhook, Hungarian Yellow Wax, Keystone Resistant Giant, King of the North, Large Cherry, Long Red Cayenne, Merrimack Wonder, New Mexico, New Mexico 6-4, Oshkosh, Rio Grande Chili, Ruby King, Sandia Chili, Sunnybrook, Sweet Banana, World Beater, Yolo Wonder, and Yolo Wonder L.

Seeds of pepper breeding lines and pepper hybrids were received from P. G. Smith, University of California, Davis, CA 95616 and included: Avelar, Agronomico-8, 1534-96-2, 2120-2-1-C, F_1 Agronomico-8 × 1333, F_1 2120-2-1 × M75, and F_2 M30 × Agronomico-8. Dr. T. A. Zitter, University of Florida, provided seed of the pepper

breeding lines 23Y and AV 23Y, as well as *Datura stramonium* L. and D. *metel* L. Tabasco seeds were obtained from the McIlhenny Co., Avery Island, Louisiana.

Seeds of *Datura meteloides* Dunal were collected around pepper-growing areas in Arizona. *Nicotiana tabacum* L. 'Xanthi', *Chenopodium amaranticolor* Coste and Reyn., *Lycopersicon esculentum* Mill. 'Bonny Best', and Anaheim chili pepper seeds were available from local sources.

Assay procedures and inoculation techniques.—The three pepper crosses and all commercial cultivars (exclusive of Anaheim and tabasco pepper) were tested only against PeMV-AzP: two separate experiments were conducted with each of these hosts, using 10 test and 5 control plants in each experiment. Individually infected California Wonder pepper plants were used as the sources of inocula.

All other hosts were tested against the eight virus isolates listed above; a minimum of five test plants per host per experiment were inoculated. Each cultivar was represented by five control plants, and all experiments were duplicated; e.g., 5 test plants/host/virus isolate with duplication consisting of two sources of inoculum: Anaheim pepper and Xanthi tobacco.

In all experiments, appropriate infected tissues were ground with 0.05 M phosphate buffer (pH 7.0; 1 ml buffer: 1 g tissue) after which 22 μm (600-mesh) Carborundum was added. Disposable plumbers' acid brushes were used to inoculate leaves.

Assays of inoculated plants were accomplished by observation of symptoms and electron microscopy. Where symptoms failed to appear, tissues were assayed on appropriate indicator plants and/or quick dip preparations were made and examined in the electron microscope. When negative results were encountered, a minimum of two additional experiments were conducted with that particular isolate and host. With certain hosts, tabasco and *C. amaranticolor*, at least six experiments were conducted, using a minimum of 10 plants per virus isolate.

Observations of infected plants (with special regard to the commercial and breeding lines of peppers) were continued during 2 mo, to note the effect of the virus on fruit and overall plant growth.

Cross protection studies.—Potato virus Y, TEV, and NC were used as the systemic viruses in cross protection experiments involving tabasco pepper. Challenge isolates consisted of PeMV-AzD and PeMV-Fla. The rationale was that systemically infected tabasco would not respond to PeMV isolates by producing local lesions, if a relationship existed between the test and the challenge virus. Challenge-inoculations were made at 5, 15, and 30 days after test-inoculations.

A special problem was presented by TEV, due to a severe wilt and death syndrome associated with tabasco infection. The problem was circumvented, however, by simply excising all leaves expressing the initial wilt symptom induced by TEV. Seven wk after the removal of such leaves, all new leaves (though limited in growth) continued to remain fully turgid and still contained virus particles, as revealed by electron microscopic examination. Such plants adequately served to receive challenge-inoculations. Intact tabasco (infected by TEV) also continued to produce limited new growth, but all new leaves wilted and eventually died.

Serology.—Antisera against PeMV-AzP and PeMV-Fla were prepared by ourselves and by D. E. Purcifull, University of Florida, Gainesville, FL, respectively. Antisera against PVY (NC 57), TEV (NC-15), and TEV (107) were provided by G. V. Gooding, Jr.

The immunodiffusion technique utilizing sodium dodecyl sulfate (SDS) (2) was used in all serological tests. Gel-patterns consisted of a center well (6 mm in diameter) and peripheral wells (5 mm in diameter) 5 mm from the center well. Antisera were placed in the center wells and antigens (prepared from 1:1 w/v tissue extracts in 0.01 M phosphate buffer, pH 7.0) were placed in the peripheral wells. Plates were kept at 22 C and examined daily for 5 days.

Electron microscopy.—Examinations were conducted with an Hitachi HS-7S electron microscope, calibrated for magnification by the use of a carbon grating replica of 2,160 lines/mm (Fullam No. 1002, Ernest F. Fullam, Schenectady, NY 12301) and an internal standard of tobacco mosaic virus. Tissue (0.5 g) to be tested was sliced in 5.0 ml of 0.01 M phosphate buffer, pH 7.0. Equal volumes of the resulting extract were mixed with phosphotungstic acid (PTA) to give a final concentration of 2% PTA.

RESULTS

Host reactions.—Table 1 outlines the contrast in susceptibility and symptom expression of selected hosts of the PeMV isolates, with PVY and TEV. Additionally, there were marked differences noted among the PeMV isolates in their individual effects on several of these hosts.

Differences in the extent of overall plant growth and the amount of fruit distortion were the variations observed with Anaheim chili. Severe stunting of this host resulted from infection with PeMV-AzD, PeMV-AzP, and PeMV-Cal. None of the other potyviruses caused the significant stunting which repeatedly characterized infection by the Arizona and California isolates. Marked differences in the nature of fruit distortion (or the lack of it) on Anaheim are illustrated in Fig. 1.

All PeMV isolates except NC induced necrotic lesions on the inoculated leaves of tabasco pepper. Following this local reaction was a rapidly spreading, systemic necrosis and an ultimate death of all plants inoculated with the Arizona and California isolates. Of the tabasco plants systemically infected with PeMV-NM, however, 20% consistently survived; i.e., secondary necrosis failed to develop, and such plants exhibited a growth comparable to healthy tabasco. An 80% rate of survival was noted consistently among those tabasco plants systemically infected by PeMV-Fla. A severe mosaic effect expressed in NC-infected tabasco; a mild, systemic necrosis occasionally would develop, but all plants continued to survive.

Chenopodium amaranticolor was the only host which responded differenctly to the two separate sources of inocula: local lesions were induced when Xanthi but not pepper was used as the inoculum source. Inhibitory effects of pepper sap have been previously reported (10, 12). This host nevertheless was not susceptible to three of the PeMV isolates even when tobacco was used as

TABLE 1. Susceptibility and symptom expression of selected indicator plants and pepper breeding lines to pepper mottle virus (PeMV) isolates from Arizona *Datura* (AzD), Arizona pepper (AzP), California (Cal), New Mexico (NM), Florida (Fla), and North Carolina (NC) in comparison with potato virus Y (PVY) and tobacco etch virus (TEV)[a]

Host species and cultivar names	Susceptibility and symptom expression in plants inoculated with virus isolate:							
	PeMV						PVY	TEV
	AzD	AzP	Cal	NM	Fla	NC		
Capsicum frutescens								
Anaheim	+	+	+	I	I	I	I	I
Tabasco	LLD	LLD	LLD	LL	LL	+	+	WD
Agronomico-8	+	+	+	M	I	M	–	–
Avelar	+	+	+	M	I	M	–	+(50%)
23Y	+	+	+	M	I	M	–	–
AV 23Y	M/+	M/+	M/+	M/+	M/+	–	–	–
2120-2-1-C	N	N	N	N	N	–	–	–
1534-96-2	–	–	–	–	–	–	–	M
Datura spp.								
D. meteloides	M	M	M	N	N	N	N	N
D. stramonium	–	–	–	–	–	–	–	+
D. metel	–	–	–	–	–	–	+	+
Nicotiana tabacum 'Xanthi'	+	+	+	M	N	N	M	+
Chenopodium amaranticolor	11	–	11	–	11	–	11	11
Tomato	N	N	N	N	N	N	I	+

[a]Symbols and abbreviations: Lack of susceptibility is indicated by (–). The remaining symbols indicate type and relative severity of reaction of susceptible plants: (+) = severe; (I) = intermediate, between mild and severe; (M) = mild; (N) = symptomless; (11) = local lesions only; (LL) = local lesions and some varying proportion of systemic necrosis and death; (LLD) = local lesions, followed by systemic necrosis and death; (WD) = wilt and death. Where a (/) separates two symbols, variation in symptom type was found on different plants.

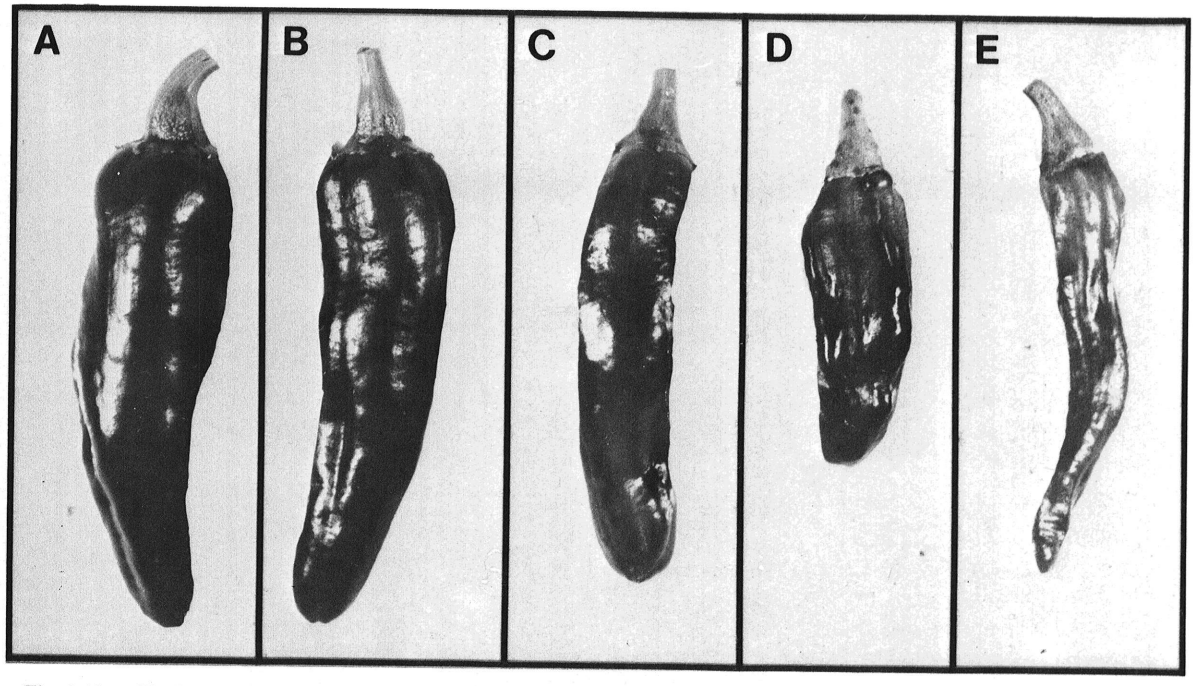

Fig. 1-(A to E). Comparison of the morphological characteristics of fruit from Anaheim pepper plants inoculated with: **A)** 0.5 M phosphate buffer control; **B)** pepper mottle virus (PeMV) Florida; **C)** tobacco etch virus (TEV); **D)** potato virus Y (PVY); and **E)** PeMV-Arizona Datura, representing PeMV-Arizona pepper, PeMV-California, PeMV-New Mexico, and North Carolina (NC).

inoculum source.

Commercial cultivars all responded to PeMV-AzP with severe foliage and fruit distortion and overall stunting with the exception of Large Cherry and Hungarian Yellow Wax: these were the only two cultivars that showed only moderate stunting and leaf distortion with near-normal fruit.

The F_2 of the cross M30 (Anaheim chili) × Agronomico-8 was found to segregate into four phenotypes when inoculated with PeMV. The four phenotypes included: (i) tolerant plants with nondistorted fruit of chili type; (ii) nontolerant plant with distorted chili fruit, (iii) as for i, except bell type fruit, and (iv) as for ii, except bell-type fruit. Through two more generations, selections were made for tolerant plants with chili type fruit (Fig. 2) and a 5-10% increase in the proportion of such individuals was noted in each generation. When progeny were not inoculated, phenotypes fell into two

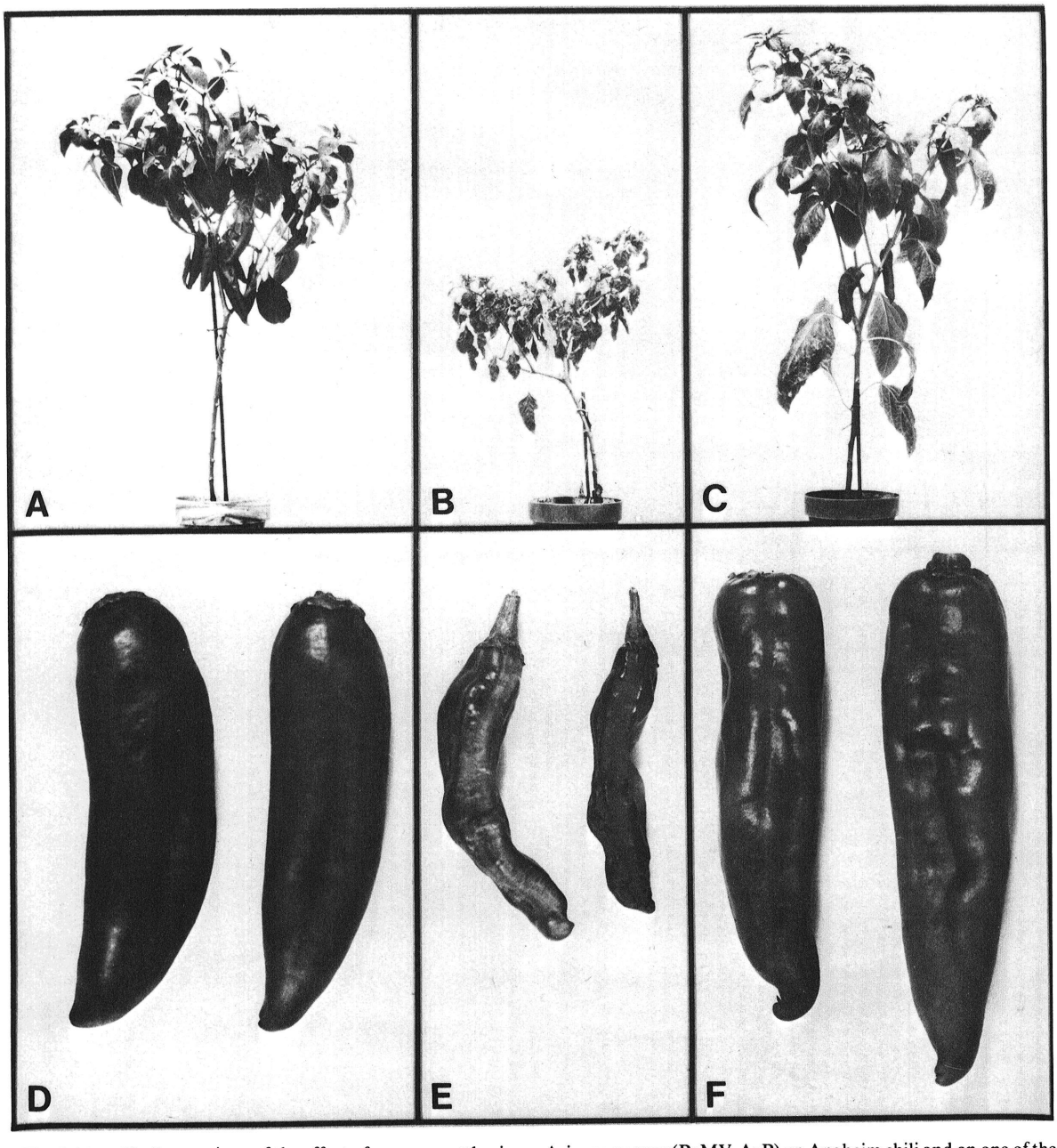

Fig. 2-(A to F). Comparison of the effect of pepper mottle virus - Arizona pepper (PeMV-AzP) on Anaheim chili and on one of the pepper crosses (F_2 M30 × Agronomico-8). **A)** Healthy Anaheim plant; **B)** Anaheim plant infected by PeMV-AzP; **C)** PeMV-AzP - infected tolerant selection from F_2 M30 × Agronomico-8; **D)** Healthy Anaheim fruit; **E)** fruit from Anaheim infected with PeMV-AzP; **F)** fruit from the selection showing tolerance for PeMV-AzP and bearing similarity to the Anaheim fruit type.

categories, chili and bell type fruit.

Cross protection.—As a result of physical and biological similarities between these potyvirus isolates, cross protection tests were conducted with tabasco pepper. Since PeMV initially generates local lesions on tabasco plants, it was used as the challenge strain in all cross protection studies.

Neither PVY, TEV, nor NC protected against either of the two PeMV isolates tested in these experiments. The pattern of lesion development on systemically infected leaves of tabasco occurred with the same frequency and at the same time as that observed on PeMV-control plants.

Serology.—All PeMV isolates, plus NC, proved identical when reacted with both PeMV antisera. None of the PeMV antigens, including NC, reacted specifically to antisera of PVY or TEV. Each of the latter two viruses reacted only to its own specific antiserum.

DISCUSSION

Based upon certain biological and physical properties one might consider all PeMV isolates, as well as TEV and NC, to be strains of PVY. Differences in host range and response fall within generally accepted limits for strain definition (3). This, coupled with the induction of similar diseases in the field, identical particle morphology, and the induction of morphologically similar characteristic inclusion bodies within the host plant (1) seem to complete the case. The problem, of course, is that no close serological relationship exists. Further chemical characterization might also show more areas of difference.

The one differential host plant most effective in distinguishing among these viruses is the pepper cultivar Tabasco. Symptoms produced by PeMV include local lesions and varying degrees of systemic necrosis (depending on the PeMV isolate), a mosaic with PVY, and the classic wilt with TEV. The NC isolate is intermediate between PVY and PeMV: it causes a mosaic symptom which is occasionally compounded with a systemic necrosis. Serologically, however, NC is related to PeMV and not to PVY. Cross protection studies in tabasco showed no relationships between PeMV, PVY, or TEV–or between NC and PeMV, despite their serological relationship. This does not mean that cross protection could not occur in some other host, since positive protection may depend on the host involved (5). Thus, the most reliable means of distinguishing between these potyviruses is serology and symptoms on tabasco pepper in that order. Any of several additional hosts listed on Table 1 can serve further to confirm the identity of these viruses.

Certain variations between isolates of PeMV were of interest. The NC isolate is apparently the only "PeMV-strain" yet known that does not induce local lesions on tabasco pepper. A separation of PeMV-NM and PeMV-Fla from the remaining three isolates is possible by the percent occurrence of rapidly spreading secondary necrosis which leads to ultimate death of tabasco. Selections AV 23Y and 2120-2-1-C further served to separate NC from all other PeMV isolates. *Chenopodium amaranticolor* reacted to only three of the PeMV isolates (PeMV-AzD, PeMV-Cal, and PeMV-Fla), but such reactions were observed only when tobacco was used as the source of inoculum. Finally and with only one exception, all PeMV isolates (including NC) had identical effects on Anaheim chili fruit. The single exception was PeMV-Fla, which had no visible effect, although electron microscopic examinations of such symptomless fruit revealed high virus concentrations.

If mixtures of the potyviruses in this study are encountered, PeMV and TEV can readily be distinguished using tabasco pepper. However, if PVY also is present in a mixture, this virus would be virtually impossible to distinguish in tabasco because of the masking effect of PeMV and/or TEV. Procedures required to identify and/or obtain pure cultures of these viruses are summarized in Table 2. To our knowledge, there is no differential host which can be used to obtain a pure culture of PVY from a mixture with TEV, but serological testing against PVY antiserum would confirm its presence. We are currently testing an Anaheim-type line of pepper which may serve as an exclusive, local lesion indicator for PVY; the results, thus far, look promising.

The Arizona and California isolates were identical, which lends support to our theory that PeMV was introduced into Arizona from southern California on chili transplants. Ten Anaheim chili samples from the Irvine Ranch in Orange County, California [the same

TABLE 2. Hosts useful for the identification and separation of certain mixtures of three potyviruses infecting peppers[a]

Mixture of virus	Hosts for	
	Identification (Symptoms on tabasco)	Separation
PeMV[b] + TEV	Local lesions (PeMV)	*Capsicum frutescens* 'Agronomico-8' (pure PeMV)
	followed by	*Datura stramonium* (pure TEV)
	Wilt (TEV)	
PeMV + PVY	Local lesions (PeMV)	*Capsicum frutescens* 'Agronomico-8' (pure PeMV)
	Mosaic (PVY) masked	*Datura metel* (pure PVY)
TEV + PVY	Wilt (TEV)	*Datura stramonium* (pure TEV)
	Mosaic (PVY) masked[c]	
PeMV + TEV + PVY	Local lesions (PeMV)	*Capsicum frutescens* 'Agronomico-8' (pure PeMV)
	followed by	*Datura stramonium* (pure TEV)
	Wilt (TEV) Mosaic (PVY) masked[c]	

[a]Please see "Materials and Methods" for more detailed descriptions of virus isolates and hosts. Refer also to Table 1 for additional identification and/or separation hosts of these viruses.

[b]Pepper mottle virus (PeMV), tobacco etch virus (TEV), and potato virus Y (PVY).

[c]Serology is required for the detection of PVY in such mixtures.

geographical area where previous isolations of "TEV" strains (4) and "PVY" strains (8) were reported to induce local lesions on tabasco pepper] were tested for virus. Four of these samples were found to be infected with pure PeMV; three of the samples contained a mixture of PeMV and TEV, and no followup was made to test for PVY; two samples were infected with pure TEV; the remaining sample was found to be virus-free.

The Irvine Ranch is located in the general area that has in past years supplied chili transplants for Arizona. For this reason and since recent work (6, 7, 8) has not substantially clarified the extent of PeMV-infected plants in California, we felt it important to establish the presence there of this virus. All virus isolates thus far obtained from both peppers and *D. meteloides* within the chili-growing regions of Arizona, near Elfrida, were identified as PeMV. It was considered likely that TEV and/or PVY might have been introduced in the same way, but neither has been found to date.

This work emphasizes again the problems of breeding for disease resistance to a closely related group of viruses. Distinct host range differences coupled with almost identical physical properties make identification very difficult. Thus, breeding and selecting for disease resistance demands that continuous attention be given to virus isolates maintained for this purpose as well as continuing attention to developments in etiology of diseases observed in the field.

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