Pepper mottle virus Causing Disease in Chile Peppers in Southern New Mexico

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ABSTRACT

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The primary pepper producing areas of southern New Mexico were surveyed to identify the viruses causing severe disease in chile peppers over a 2-year period. The survey included weeds commonly found in and around pepper fields. Using indirect enzyme-linked immunosorbent assay (ELISA), *Pepper mottle virus* (PepMoV) was associated with plants showing mosaic and distortion of foliage and fruit deformation. PepMoV and *Cucumber mosaic virus* (CMV) were determined based on ELISA to be infecting chile peppers and weeds singly or in combination. Four perennial plant species were infected with PepMoV and CMV, including *Solanum elaeagnifolium* (silverleaf nightshade), *Convolvulus arvensis* (field bindweed), and *Chamysuraces* sp. (small groundcherry), which had not previously been identified as hosts for PepMoV. Some peppers and weeds surveyed were also infected at a lower level by several other plant viruses.

Additional keywords: ELISA, weed hosts

Viral diseases in field-grown pepper (*Capsicum annuum* L.) plants are a major concern for the pepper growers and the processing industry of southern New Mexico. Viruses infecting peppers are known to induce deformation of fruit and reduce yields (1,3,6,10,11). Pepper growing areas in Arizona (6), California (1), Florida (11), Georgia (3), and Texas (10) have been affected by several viruses, including *Cucumber mosaic virus* (CMV), *Pepper mottle virus* (PepMoV), *Tobacco etch virus* (TEV), *Tomato spotted wilt virus* (TSWV), *Alfalfa mosaic virus* (AIMV), and *Potato virus Y* (PVY). Mixed infections of viruses infecting peppers are frequently reported. Abdalla et al. (1) detected seven viruses in single or mixed infections in California peppers, with CMV and PepMoV present in most plants. In northeastern Georgia, TEV and CMV were the predominant viruses in pepper fields (3). Thus, the distribution of viruses infecting peppers is variable and dependent on field location.

Pepper plants with fruit deformation and uneven color, foliage distortion, mosaic, or mild mottle became a concern to producers and processors in southern New Mexico during 1992. The incidence of diseased plants appeared higher than in previous years with apparent differential symptom severity among varieties, and some pepper fields approaching 100% symptomatic plants. The foliage of plants in a given pepper field displayed a range of symptoms suggesting that more than one virus was infecting plants in the field. Nelson and Wheeler (6) reported one isolate of PepMoV from the Hatch area in New Mexico in 1978. Moreover, preliminary reports have indicated the presence of several other viruses infecting peppers in southern New Mexico (5).

This study documents the occurrence of PepMoV and CMV in field-grown pepper plants in southern New Mexico. Additionally, the presence of virus-infected perennial and annual weeds as hosts is confirmed.

MATERIALS AND METHODS

Virus isolates and mechanical transmission. CMV-S (subgroup II) and CMV-SD2 (subgroup I) were previously described (8). TEV-Ox was obtained from W. Daugherty (Oregon State University, Corvallis), PVY was obtained from J. L. Sherwood (Oklahoma State University, Stillwater) (9), and PepMoV-Ca was obtained from J. A. Dodds (University of California, Riverside). PepMoV #B725 was isolated from sacred datura (*Datura innoxia* Mill.), a perennial weed found near a pepper field north of Las Cruces, NM. Viruses were maintained in dried tobacco (*Nicotiana tabacum* L. 'Xanthi nc') tissue

at 4°C, and in greenhouse-grown tobacco plants. Viruses used as controls and selected field virus isolates (determined using enzyme-linked immunosorbent assay [ELISA]) were inoculated on seedlings of jimsonweed (*D. stramonium* L.), *Chenopodium amaranticolor* Willd., chile pepper (*Capsicum annuum* L., cv. NM 64) and tobacco, by rubbing buffered (0.02 M potassium phosphate, pH 7.0, 0.1% sodium thioglycolate, 0.3% sodium diethyldithiocarbamate) sap extract on 300-mesh Carborundum-dusted leaves.

Antisera. The PepMoV, PVY, and CMV (subgroup I) antisera was supplied by B. Falk (University of California, Davis), TEV antiserum was provided by Agdia, Inc. (Elkhart, IN), and the CMV (subgroup II) and AIMV antisera were obtained from American Type Culture Collection (Rockville, MD). The immunoglobulin (IgG) fraction from each antiserum was prepared using an Acti-Disk separation and purification cartridge (FMC, Rockland, ME). CMV subgroup I and II antisera were combined for serological tests. Selected pepper and weed samples collected in 1992 were sent to R. Jordan, USDA, Beltsville, MD for confirmation of PepMoV infection using monoclonal antibody ELISA.

Collection of pepper and weed samples. Plant samples were collected from 37 pepper farms primarily from the Deming, Las Cruces, and Hatch areas of southern New Mexico during 1992 and 1993. The pepper samples included leaves from young shoots with or without distinct symptoms of viral infection. Samples from common weeds were collected within areas bordering pepper fields and in nearby irrigation ditches. Weed samples were leaves with or without symptoms. Samples were stored at 4°C for 1 or 2 days prior to serological testing.

Sera cross-absorption. Antisera were cross-absorbed with healthy plant proteins using a modification of Ball et al. (2). Healthy plant leaf tissue (100 mg) was ground in 10 ml of phosphate-buffered saline (PBS) (0.1 M, pH 7.4), and centrifuged at 12,000 × g for 1 min. The supernatant was collected and 20 mg IgG was added to 1 ml, and incubated at room temperature for 1 to 2 h. After centrifugation at 12,000 × g for 1 min, the mixture was diluted in 10 ml of PBS plus 0.05% Tween 20, 2% polyvinylpyrrolidone 40,000 and 0.2% ovalbumin.

Indirect ELISA. Indirect plate-trapped ELISA (4) was used to detect viruses in plant samples. Each sample was tested

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twice. Leaf samples were ground in coating buffer (0.05 M carbonate, 0.02% sodium azide, pH 9.6) at 1:10 (wt/vol) and clarified by centrifugation at 12,000 × g for 1 min. The antigen was further diluted in coating buffer to 1:10 (vol/vol) for PepMoV detection, and 1:1,000 (vol/vol) for CMV, PVY, and TEV at 200 µl/well and incubated at 4°C overnight. After washing the plate, 200 µl of cross-absorbed IgGs were added per well and plates were incubated at 4°C overnight. Plates were washed and alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma, St. Louis, MO) was added at a 1/10,000 dilution and incubated 2 h at 37°C. *p*-Nitrophenyl phosphate (200 µl/well) was added at 1 mg/ml and incubated at room temperature for 30 to 60 min for color development. Readings of reactions were carried out after 1 h at 405 nm in an EL-312e Microplate Bio-Kinetics Reader (Bio-Tek Instruments, Inc.). Samples were considered positive when absorbance readings (A_{405}) of sample wells were greater than three times the mean value of three healthy controls. This relationship was determined by ELISA sensitivity assays of chile infected with CMV, PVY, PepMoV, or TEV that was maintained in the greenhouse.

RESULTS AND DISCUSSION

There were 685 pepper (Table 1) and 336 weed samples (Table 2) tested for virus infection using ELISA. During 1992, 42.6% (Table 1) of the pepper samples were positive for PepMoV and/or CMV, with 57.1% of those plants infected with PepMoV, 35.7% infected with a mixture of PepMoV and CMV, and only 7.1% infected with CMV alone. During 1993, the percentage of infected pepper samples was 45.6% (Table 1). In 1993, 89.7% of the ELISA-positive pepper plants were infected with PepMoV, 0.9% were infected with a mixture of PepMoV and CMV, and only 0.4% were infected with CMV.

The percentage of weeds infected during 1992 and 1993 was 74.6 and 68%, respectively (Table 2). These values are higher than those found for pepper samples. The majority of weed samples from 1992 were coinfecting by PepMoV and CMV (79.7%, Table 2), whereas in 1993, the percentage of doubly-infected weed samples was 45.4% (Table 2).

The perennial plants silverleaf nightshade (*Solanum elaeagnifolium*), small ground cherry (*Chamysuraces* sp.), sacred datura (*D. innoxia*), field bindweed (*Convolvulus arvensis*), and the annual plants Wrights ground cherry (*Physalis wrightii*

Gray), were infected with PepMoV and/or CMV using ELISA (Table 2) and exhibited typical symptoms on indicator hosts (data not shown). These plants are commonly found in and near pepper fields in southern New Mexico. *D. innoxia* was found only in fence rows, while small *Chamysuraces* sp. plants were found only in irrigation ditches. *S. elaeagnifolium* and *C. arvensis* plants, were extensively sampled because these plants were ubiquitous in their distribution and have the highest populations in the areas sampled. Several plant species collected included mesquite (*Prosopis glandulosa* Torr.), lambsquarters (*Chenopodium album* L.), saltbush (*Atriplex* sp.), whorled milk weed (*Asclepias subverticillata* (Gray) Vail), and Russian thistle (*Salsola tragus* Torner ex L.), were not infected.

D. meteloides Dunal, a perennial, has been described as the main overwintering host for PepMoV in Arizona (7), while *D. metel* L. and *D. stramonium* are not susceptible to this virus (6). In this study, PepMoV infected several *D. innoxia* plants near pepper fields and in three other perennial plants, *S. elaeagnifolium*, *Chamysuraces* sp., and *C. arvensis*. These perennials may serve as overwintering sources of PepMoV in southern New Mexico and as overwintering habitat for the aphid vectors of the virus. Several species of aphids, including *Myzus persicae* Sulz., transmit PepMoV in a nonpersistent manner (7).

During April and May of 1993, samples collected from actively growing weeds had a high incidence of PepMoV and CMV (Table 3). This suggests that the source of virus inoculum was already present when the peppers germinated during those months. The varying incidence of virus-infected plants among pepper farms suggests that those severely affected might be nearer to the virus inoculum source and/or that PepMoV is endemic in perennial plants in those areas. Seed transmission of PepMoV has not been documented (7).

When selected ELISA-positive samples were tested for virus infection using indicator species, PepMoV produced a mild mottle on tobacco plants, whereas CMV or a mixture of CMV plus PepMoV produced an obvious mosaic on tobacco plants. CMV produced mosaic in *D. stramonium* and local lesions in *Chenopodium amaranticolor*. The PepMoV isolates from southern New Mexico induced no symptoms in these two hosts, which was similar to the responses of the PepMoV isolate described previously from Hatch, NM (6), and contrasted with the response of the PepMoV strain from California, which induced local lesions in *Chenopodium amaranticolor* (6).

ELISA results indicated the presence of TEV and PVY in pepper and weed samples, however, not all plants were tested for these viruses. In 1992, both viruses were detected in peppers collected from Deming and Las Cruces, NM, and in

Table 1. Occurrence of *Cucumber mosaic virus* (CMV) and *Pepper mottle virus* (PepMoV) in pepper samples collected in southern New Mexico during 1992 and 1993

Locations where plants were collected	Year	Number of plants assayed	Number of plants infected with virus		
			CMV	PepMoV	CMV + PepMoV
Deming	1992	60	0	36	2
	1993	234	0	119	1
Las Cruces, Vado	1992	52	4	4	27
	1993	54	0	35	0
Hatch, Rincon, Garfield	1992	85	2	8	1
	1993	198	1	65	1
Hobbes	1993	2	0	1	0

Table 2. Occurrence of *Cucumber mosaic virus* (CMV) and *Pepper mottle virus* (PepMoV) in weed samples collected in southern New Mexico during 1992 and 1993

Weed species	Year	Number of plants assayed	Number of plants infected with virus		
			CMV	PepMoV	CMV + PepMoV
<i>Solanum elaeagnifolium</i> Cav.	1992	77	13	2	34
	1993	123	4	27	51
<i>Chamysuraces</i> sp.	1992	5	0	0	5
	1993	28	0	18	0
<i>Datura innoxia</i> Mill.	1992	1	0	1	0
	1993	4	1	3	0
<i>Convolvulus arvensis</i> L.	1992	31	2	1	24
	1993	53	0	23	14
<i>Physalis wrightii</i> Gray	1992	12	0	0	12
	1993	2	0	2	0

Table 3. Occurrence of *Pepper mottle virus* and *Cucumber mosaic virus* in plant samples collected in the Deming, NM area during 1993

Plant assayed	Number infected/number tested					
	April	May	June	July	August	October
<i>Solanum elaeagnifolium</i> Cav.	59/59	6/6	0/0	6/11	0/0	0/24
<i>Convolvulus arvensis</i> L.	33/37	6/6	0/0	0/0	0/0	0/0
<i>Chamysuraces</i> sp.	1/11	13/13	0/0	4/4	0/0	0/0
Pepper	0/0	0/0	0/29	18/75	48/52	55/77

1993, both viruses were detected from Deming. In 1992 and 1993, TEV and PVY were detected from *S. elaeagnifolium* and *C. arvensis*, while in 1992, the viruses were detected from these two weed hosts and from *Chamysuraces* sp. and *Physalis wrightii*.

All 14 pepper samples and one weed sample (*D. innoxia*) tested positive for PepMoV using monoclonal antibodies. The results for two of the pepper samples analyzed using monoclonal antibodies indicated the presence of variability among the PepMoV isolates and the presence of other potyviruses in the pepper samples.

Pepper samples collected in 1993 near alfalfa fields gave positive ELISA results for AIMV (data not shown). A few pepper samples showed symptoms characteristic of those caused by TSWV such as chlorotic concentric rings on pods and leaves, but they were not assayed for that virus. In addition, stunted peppers were found with curled leaves characteristic of infection with *Beet curly top virus*, but no confirmatory analyses were conducted. Peppers not showing viral symptoms were not positive using ELISA for any virus tested.

Pepper plants showing a range of viral symptoms were observed during our field sampling. The most common viral symptoms observed were distorted fruit with uneven color and distorted foliage with mosaic or mild mottle. PepMoV was con-

sistently detected in pepper plants showing these symptoms, suggesting it had an active role in causing these symptoms. Other symptoms could have been caused by other viruses or by synergistic interactions among the viruses. PepMoV appears to have been the most significant factor in the 1992 to 1993 viral epidemic of pepper in southern New Mexico.

The presence of PepMoV in common perennial and annual weeds found near pepper fields and the yearly cultivation of susceptible chile plants suggest that the virus might continue to impact chile production. PepMoV continues to be detected infecting chile in southern New Mexico fields each year, although the incidence is considerably less than epidemic.

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