Management of Tomato Mosaic Virus in Hydroponically Grown Pepper (*Capsicum annuum*)

KRISTIN G. PATEGAS, Nursery Supervisor, Walt Disney World Co., Lake Buena Vista, FL 32830; ANDREW C. SCHUERGER, Supervisor and Technical Specialist I, The Land, EPCOT Center, Lake Buena Vista, FL 32830; and CARL WETTER, Professor, Department of Botany, University of Saarland, D-6600 Saarbrucken, West Germany

ABSTRACT

Pategas, K. G., Schuerger, A. C., and Wetter, C. 1989. Management of tomato mosaic virus in hydroponically grown pepper (*Capsicum annuum*). Plant Disease 73: 570-573.

A tobamovirus causing severe systemic necrosis of hydroponically grown pepper plants (*Capsicum annuum* 'Hungarian Wax') was identified by host plant reactions and immunodiffusion tests as tomato mosaic virus (ToMV) (EPCOT isolate). Twenty-eight pepper cultivars were screened for resistance to this isolate. Anaheim TMR 23 and Rio Grande Gold were the most resistant cultivars; plants exhibited necrotic local lesions and abscission of inoculated leaves. Golden Belle, Hidalgo, Super Stuff, Tamcascabella, and Tam Mild Jalapeno-1 were the most susceptible cultivars; plants exhibited foliar chlorosis and severe systemic necrosis, which resulted in the death of the plants within 21 days. Seven sanitizing solutions were tested for their ability to reduce ToMV transmission between plants via pruning shears. Symptoms developed in 70% of control plants pruned with ToMV-contaminated, chemically untreated shears. The transmission was reduced, with the virus being transmitted to 3, 17, 22, and 39% of inoculated plants, when the shears were treated with 10% trisodium phosphate, a combination of 0.26% NaOCl and 0.01% Ivory Liquid, 0.26% NaOCl, and 0.4% RD20, respectively. ToMV transmission was not significantly reduced when the pruning shears were treated with 0.01% Ivory Liquid, sterile deionized water, or 70% ethanol.

Additional keywords: aeroponic, host range

In the fall of 1985, a severe leaf and stem necrosis occurred on hydroponically grown pepper plants (*Capsicum annuum* L. 'Hungarian Wax') in greenhouses at The Land, EPCOT Center, Lake Buena Vista, Florida. Several weeks after the initial outbreak on peppers, chlorotic mosaic and leaf deformity symptoms were observed on nearby tomato plants (*Lycopersicon esculentum* Mill. 'Sweet 100'), and latent infections were detected in tomato plants growing in an adjacent greenhouse. By January 1986, a chlorotic mottle was present on all pepper and tomato crops in the greenhouses. Epidermal leaf strips of chlorotic pepper leaves that were stained with azure A and heat-treated (7) contained hexagonal plates, and a preliminary host range study indicated that tomato mosaic virus (ToMV), henceforth called the EPCOT isolate, was present in these mottled plants. In an effort to control the spread of the virus in The Land greenhouses, infected plant material was removed, and all greenhouse and plant support surfaces were cleaned with a sanitizing solution of 0.26% sodium hypochlorite and 0.01% Ivory Liquid (Procter and Gamble, Cincinnati, OH). A single application of the sanitizing solution was not effective in eradicating the disease, and sporadic outbreaks continued through the spring of 1986.

The Land, at EPCOT Center, displays 50–60 food crops in 0.6 ha of greenhouses. The management of a mechanically transmitted viral disease within the highly intercropped and confined spaces at The Land is particularly difficult because plants are handled several times a day, to maximize visual qualities. Research was initiated to screen pepper cultivars for resistance to ToMV and to test the efficacy of chemicals for surface disinfestation of pruning shears. Portions of this study have been previously published (16).

Accepted for publication 28 January 1989.

MATERIALS AND METHODS

Identification. Epidermal leaf strips from infected pepper plants were stained with a combination of calcomine orange and Luxol brilliant green and with azure A as described by Christie and Edwardson (7). Samples were observed with a Nikon Optiphot compound microscope. Leaf dips were prepared from systemically infected pepper plants, *C. annuum* 'Hungarian Wax.' Leaf extracts for electron microscopy were placed on Formvar (Ladd Research Industries, Burlington, VT) and carbon-coated copper grids, washed with 0.05 M phosphate buffer (pH 7.0), cleared with bacitracin, and negatively stained with 2% uranyl acetate. The grids were air-dried and then observed with a Hitachi H-600 transmission electron microscope.

Diagnostic species for host range studies were selected from the literature (9,14,22). Leaf extracts for manual inoculations were prepared by triturating systemically infected Hungarian Wax pepper leaves in 0.2 M phosphate buffer (pH 7.0). Two young true leaves of 10 pepper plants (6 wk old) were dusted with 320-grit Carborundum and rubbed with a sterile cheesecloth pad saturated with inoculum. The plants were observed for 14 days. Host range tests were conducted at least twice. The mean temperature and relative humidity were 25.8 C and 63.0%, respectively, for the duration of the host range study.

Antisera preparation and agar gel immunodiffusion tests were conducted on slides as previously described by Wetter et al (22). Our experimental virus was tested serologically with antisera of ToMV Dahlemense strain, described by Wang and Knight (20), tobacco mosaic virus (TMV) common strain U1, tobacco mild green mosaic virus, pepper mild mottle virus, bell pepper mottle virus, and Odontoglossum ringspot virus. The type strain and antiserum of ToMV Dahlemense were obtained from H. H. Murakishi; all other type strains and antisera were from laboratory stocks (C. Wetter) and were described previously (23).

Resistance of pepper cultivars. Fourteen pepper cultivars were chosen for their reported resistance to TMV (12,19,21) or ToMV (15,17,21,22), 10 other cultivars were selected on the basis of their listings in commercial seed catalogs for resistance to TMV, and four cultivars were selected because of their horticultural traits (Table 1). Two lower true leaves of each of four 6-wk-old plants were inoculated as described for the host range study. Hungarian Wax pepper plants were inoculated for each test as a susceptible check. Plants of the 28 pepper cultivars were inoculated at the same time, and the experiment was conducted four times.

The resistance of selected pepper cultivars to the virus was rated after 21 days on a scale of 1–4 based on the development of symptoms (Table 1), similar to the scales used by Holmes (11) and Rast (17) for TMV. Values were subjected to an analysis of variance (*F*

test), and the means were statistically ranked by Fisher's least significant difference test ($P = 0.05$).

Sanitation of pruning shears. Sterile deionized water, 0.26% NaOCl (5% household liquid bleach; Purex Corp., Phoenix, AZ), 0.01% Ivory Liquid, 0.4% RD20 (*N*-alkyldimethylbenzylammonium chloride; R.D. & Associates, Inc., Pomona, CA), 70% ethanol, 10% (w/v) trisodium phosphate (Na_3PO_4), and a combination of 0.26% NaOCl and 0.01% Ivory Liquid were compared for their effectiveness in reducing the transmission of the EPCOT isolate of ToMV by contaminated pruning shears. Pruning shears were dipped in triturated leaf extracts prepared as described for the host range study. The virus-contaminated shears were used to inoculate five 6-wk-old Hungarian Wax pepper plants by cutting off four lower true leaves and four young terminal leaves on each plant. The first plant inoculated served as a control to confirm virus infectivity. Following the inoculation of the first plant, the contaminated pruning shears were dipped in one of the sanitizing solutions in a manner similar to normal greenhouse practice at The Land (the blades were dipped in the solution, agitated for 5–10 sec, and not rinsed). The chemically treated shears were then used to prune four additional pepper plants in a similar manner. A chemically untreated, virus-contaminated set of pruning shears was used to inoculate control plants. The treatments were arranged in a completely randomized design in a 7.5- × 3.2-m greenhouse. Each treatment was replicated three times, and the experiment was conducted eight times, for a total of 24 tests per treatment. The mean temperature and relative humidity were 23.6 C and 78.0%, respectively, for the duration of the experiment.

At 14 days, pepper plants were assigned 0 if they did not exhibit symptoms typical of Hungarian Wax infected with ToMV and 1 if they did exhibit symptoms. The values were transformed to arc sines and subjected to Cochran's Q test for analysis. The means were statistically ranked by the McNemár test for significant changes ($P = 0.001$).

Environmental data collection. Data on relative humidity and air temperature were collected and averaged hourly by a Campbell Scientific CR7X Measurement and Control System (Campbell Scientific, Inc., Logan, UT). Environmental data were processed using The Land's database management system (13).

RESULTS

Identification. Hexagonal crystals, like those described for tobamoviruses (7), were observed when epidermal leaf strips from typically mottled, hydroponically grown *C. annuum* 'Hungarian Wax' plants were stained and heated with azure A. They were not observed when epidermal leaf strips were stained but not heated with azure A. Inclusion bodies of other virus groups were not observed following the treatment of epidermal leaf strips with calcomine orange and Luxol brilliant green. Rigid rods were observed by electron microscope in negative-stained leaf extract preparations. No other virus particle types were observed. The thermal inactivation of crude sap from infected *C. annuum* leaves was 90–95 C.

The results of the host range study were consistent with the literature for ToMV (9,14,22) except for two plant species. Symptoms were not observed on *Ocimum basilicum* L., which has been reported to be a chlorotic local lesion host of ToMV (22), and local lesions were observed on *Physalis floridana* L. without the development of systemic symptoms. *P. floridana* has been reported to be a diagnostic species for ToMV, with systemic symptoms (9).

In one-way agar gel slide tests, the EPCOT isolate was evaluated for its serological relationships with six tobamoviruses. It reacted with the ToMV Dahlemense isolate, with a complete fusion of precipitin lines, in the presence of ToMV Dahlemense antiserum. However, the EPCOT isolate produced spurs when placed next to any of the other tobamovirus type strains and their homologous antisera. From this evidence, we conclude that the EPCOT isolate is ToMV.

Resistance of pepper cultivars. Cultivars with high resistance to the EPCOT isolate of ToMV included Anaheim TMR 23, Rio Grande Gold, and Tam Mild Chile-1, which had ratings of 1.0–1.3 (Table 1). Cultivars with ratings of 1.5–2.3 were considered moderately resistant and included Bell Boy, California Wonder 300, Early Thickset, Emerald Giant, Keystone

Table 1. Reactions of hydroponically grown pepper cultivars mechanically inoculated with the EPCOT strain of tomato mosaic virus

Cultivar	Source[x]	Highly resistant[y,z]	Moderately resistant	Moderately susceptible	Highly susceptible
Anaheim TMR 23	RS	1.0 a	—	—	—
Rio Grande Gold	TAES	1.0 a	—	—	—
Tam Mild Chile-1	TAES	1.3 ab	—	—	—
Tambel-1	TAES	1.5 b	—	—	—
Bell Boy	RS	—	2.0 c	—	—
California Wonder 300	P	—	2.0 c	—	—
Early Thickset	Park	—	2.0 c	—	—
Emerald Giant	NK	—	2.0 c	—	—
Keystone Resistant Giant 4	P	—	2.0 c	—	—
NVH 3053	NK	—	2.0 c	—	—
Sirono	RS	—	2.0 c	—	—
Tambel-2	TAES	—	2.0 c	—	—
Yolo Wonder B	P	—	2.0 c	—	—
Jupiter	NK	—	2.3 cd	—	—
P324	SG	—	2.3 cd	—	—
Resistant Giant	RS	—	2.3 cd	—	—
Yolo Wonder	Park	—	2.3 cd	—	—
Bell Captain	P	—	—	2.5 de	—
FLVR-2	FF	—	—	2.5 de	—
Lamuyo	RS	—	—	2.8 ef	—
Mayata	RS	—	—	2.8 ef	—
Calumet	RS	—	—	3.0 f	—
Golden Belle	HM	—	—	—	4.0 g
Hidalgo	TAES	—	—	—	4.0 g
Hungarian Wax	HM	—	—	—	4.0 g
Super Stuff	SS	—	—	—	4.0 g
Tamcascabella	TAES	—	—	—	4.0 g
Tam Mild Jalapeno-1	TAES	—	—	—	4.0 g

[x]FF = Florida Foundation Seed, Greenwood, FL; HM = Harris Moran Seeds, Salinas, CA; NK = Northrup King, Gilroy, CA; P = Petoseed, Saticoy, CA; Park = Park Seed, Greenwood, SC; RS = Royal Sluis, Enkhuizen, Holland; SG = Sluis & Groot, Salinas, CA; SS = Stokes Seeds, Buffalo, NY; TAES = Texas Agricultural Experiment Station, Weslaco.

[y]Pepper cultivar ratings were based on symptom development of 16 plants after 21 days of observation as follows: 1 = necrotic local lesions and abscission of inoculated leaves developed within 48 hr, and no further systemic symptoms were observed; 2 = necrotic local lesions developed on inoculated leaves, and systemic leaf necrosis developed on uninoculated leaves, but no systemic stem necrosis was observed; 3 = symptoms were similar to rating 2, but systemic necrosis of the main stem was also present; 4 = chlorotic local lesions developed on inoculated leaves after 48 hr, and systemic chlorosis, necrosis, and defoliation resulted in the death of the plant.

[z]Values followed by the same letter are not significantly different according to Fisher's least significant difference test ($P = 0.05$).

Resistant Giant 4, NVH 3053, Sirono, Tambel-1, Tambel-2, and Yolo Wonder B. Cultivars with a rating of 2.5 or higher were considered too susceptible to the EPCOT isolate of ToMV to be usable in The Land greenhouses.

Sanitation of pruning shears. The EPCOT isolate of ToMV was successfully transmitted by contaminated pruning shears to Hungarian Wax pepper plants (Table 2). Typical systemic symptoms developed in 70% of the control plants, pruned with virus-contaminated, chemically untreated shears. The transmission was reduced, with the virus transmitted to 3, 17, 22, and 39% of the inoculated plants, when contaminated shears were treated prior to pruning with solutions of 10% Na_3PO_4, a combination of 0.26% NaOCl and 0.01% Ivory Liquid, 0.26% NaOCl, and 0.4% RD20, respectively (Table 2). These reductions were significant at $P = 0.001$. No significant reductions in virus transmission were observed when the shears were treated with 0.01% Ivory Liquid, sterile deionized water, or 70% ethanol.

DISCUSSION

Many pepper cultivars have been reported to be resistant to TMV (12,19,21), but few have been reported with resistance to ToMV (15,17,21,22). In our studies only the EPCOT isolate of ToMV was used for the pepper resistance trial. In addition, the evaluation period for disease development in peppers was only 21 days and may not reflect field resistance to ToMV. Plant age and environmental conditions have been reported to affect the development of diseases caused by ToMV (1,2) and TMV (12). Therefore, pepper cultivars with ratings of 1.0–2.3 should be reevaluated for ToMV resistance under field conditions.

Several studies have reported the success of disinfestation of equipment surfaces in managing tobamoviruses on tomato crops (1,3,10) and peppers (8). Brock (6) reported that Na_3PO_4 inactivates tobamoviruses on hands and tools. Consistent with these reports, the 10% Na_3PO_4 solution was the most successful treatment in our studies, reducing ToMV transmission so that the virus was transmitted by pruning shears to only 3% of the inoculated plants. However, the inactivation of ToMV on pruning equipment must be 100% effective to be useful in greenhouse or nursery operations. The intensive handling of plant material in these facilities would result in unacceptable crop losses if even one plant became infected. Longer treatment times in the sanitizing solution could be used to decrease transmission rates further. Preliminary experiments using 12 pepper plants per treatment showed that treating contaminated pruning shears for 10 min in 10% Na_3PO_4 or a combination of 0.26% NaOCl and 0.01% Ivory Liquid reduced transmission rates of the EPCOT isolate of ToMV to 0 (data not shown). Future experiments will continue in this area.

The initial source of ToMV inoculum at The Land was not identified, but mechanical transmission into the greenhouses by workers or visitors was suspected. ToMV can persist on workers' clothing (4,5), greenhouse structures (4), and horticultural tools and workers' hands (1), and it can be readily transmitted by contaminated seed (3,5). Contaminated seed, however, was not suspected as the primary source of inoculum, because the same seed lots of tomato and pepper were used throughout the fall of 1985 and the spring of 1986 without a consistent correlation with the occurrence of the disease.

Secondary spread of the virus was believed to be due to mechanical transmission through contaminated pruning shears, ladders, and workers' hands. A single treatment of ladders and horticultural tools with 0.26% NaOCl plus 0.01% Ivory Liquid and the removal of plants exhibiting disease symptoms were not successful in eradicating the disease. Broadbent and Fletcher (4) demonstrated that ToMV could survive up to 9 wk on greenhouse structures coated with new glossy paint. The greenhouses at The Land are coated with plastic, and virus particles may have survived on these surfaces, providing the inoculum that was responsible for recurrent outbreaks of ToMV on pepper and tomato plants. Another possible source of secondary inoculum may have been the recirculating nutrient solution used in the aeroponic growing systems. Roberts (18) demonstrated that TMV, tomato bushy stunt, and potato virus X can infect hydroponically grown tomato plants when leaf sap extracts from virus-infected plants are added to the nutrient solution. Tests with The Land's nutrient solution were inconclusive, and future research will investigate the possibility of virus transmission in hydroponic solutions. Outbreaks of the disease were eliminated only after several extensive surface washings of the greenhouse structures and horticultural equipment and treatment of the nutrient solution recirculating system with 0.26% NaOCl and 0.01% Ivory Liquid.

Table 2. Incidence of virus symptoms on *Capsicum annuum* 'Hungarian Wax' when pruning shears infested with the EPCOT strain of tomato mosaic virus (ToMV) were treated prior to pruning

Treatment	Range percent infection[w]	Percentage of infected plants[x,y]
10% Na_3PO_4	0–25	3 a
0.26% NaOCl and 0.01% Ivory Liquid	0–25	17 bc
0.26% NaOCl	0–50	22 bcd
0.4% RD20	8–67	39 cd
0.01% Ivory Liquid	25–83	51 de
Sterile deionized water	42–92	71 e
70% Ethanol	25–100	71 e
Control[z]	42–92	70 e

[w] Range percent infection is the range of the percentages of inoculated plants exhibiting symptoms in eight replicates.

[x] Mean obtained from eight tests, with 12 plants per test pruned with shears treated with the sanitizing solutions. A total of 96 plants were pruned for the experiment.

[y] Values followed by the same letter are not significantly different according to the McNemar test for significant changes ($P = 0.001$). The data were transformed to arc sines prior to statistical analysis.

[z] The control treatment consisted of pruning plants with ToMV-contaminated, chemically untreated pruning shears.

ACKNOWLEDGMENTS

We would like to thank M. S. Elliott for assistance with the electron microscopy; B. Villalón for supplying pepper seed; and R. H. Converse, D. J. Mitchell, and F. W. Zettler for editorial comments on the manuscript.

LITERATURE CITED

1. Broadbent, L. 1963. The epidemiology of tomato mosaic. III. Cleaning virus from hands and tools. Ann. Appl. Biol. 52:225-232.
2. Broadbent, L. 1965. The epidemiology of tomato mosaic. VIII. Virus infection through tomato roots. Ann. Appl. Biol. 55:57-66.
3. Broadbent, L. 1965. The epidemiology of tomato mosaic. XI. Seed-transmission of TMV. Ann. Appl. Biol. 56:177-205.
4. Broadbent, L., and Fletcher, J. T. 1963. The epidemiology of tomato mosaic. IV. Persistence of virus on clothing and glasshouse structures. Ann. Appl. Biol. 52:233-241.
5. Broadbent, L., and Fletcher, J. T. 1966. The epidemiology of tomato mosaic. XII. Sources of TMV in commercial tomato crops under glass. Ann. Appl. Biol. 57:113-120.
6. Brock, R. D. 1952. The use of trisodium phosphate as an inactivating agent for plant viruses. J. Aust. Agric. Sci. 18:41-43.
7. Christie, R. G., and Edwardson, J. R. 1977. Light and electron microscopy of plant virus inclusions. Fla. Agric. Exp. Stn. Bull. 9. 155 pp.
8. Demski, J. W. 1981. Tobacco mosaic virus is seedborne in pimiento peppers. Plant Dis. 65:723-724.
9. Feldman, J. M., and Oremianer, S. 1972. An unusual strain of tobacco mosaic virus from pepper. Phytopathol. Z. 75:250-267.
10. Gooding, G. V., Jr. 1975. Inactivation of tobacco mosaic virus on tomato seed with trisodium orthophosphate and sodium hypochlorite. Plant Dis. Rep. 59:770-772.
11. Holmes, F. O. 1937. Inheritance of resistance to tobacco-mosaic disease in the pepper. Phytopathology 27:637-642.
12. Igwegbe, E. C. K., and Ogungbade, O. K. 1985.

Evaluation of pepper cultivars under greenhouse conditions for resistance to a defoliation strain of tobacco mosaic virus. Plant Dis. 69:899-900.
13. Muller, E. R., and Harriott, J. T. 1984. A data management system for multiple crops. Pap. 84-5518. Am. Soc. Agric. Eng. Winter Meet.
14. Murakishi, H. H., and Honma, S. 1960. A strain of tobacco mosaic virus associated with a wilt and stem necrosis of mature, field-grown bell pepper. Plant Dis. Rep. 44:550-551.
15. Paludan, N. 1982. Virus attack in Danish cultures of sweet pepper (*Capsicum annuum* L.) specially concerning tobacco mosaic virus. Acta Hortic. 127:65-75.
16. Pategas, K. G., and Schuerger, A. C. 1986. Evaluation of resistance of *Capsicum annuum* cultivars to a tobamovirus isolated from aeroponically grown peppers in Florida. (Abstr.) Phytopathology 76:1121.
17. Rast, A. T. B. 1982. Resistance of *Capsicum* species to tobacco, tomato, and pepper strains of tobacco mosaic virus. Neth. J. Plant Pathol. 88:163-169.
18. Roberts, F. M. 1950. The infection of plants by viruses through roots. Ann. Appl. Biol. 37:385-396.
19. Villalón, B. 1981. Breeding peppers to resist virus diseases. Plant Dis. 65:557-562.
20. Wang, A. L., and Knight, C. A. 1967. Analysis of protein components of tomato strains of tobacco mosaic virus. Virology 31:101-106.
21. Wetter, C. 1984. Serological identification of four tobamoviruses infecting pepper. Plant Dis. 68:597-599.
22. Wetter, C., Conti, M., Altschuh, D., Tabillion, R., and van Regenmortel, M. H. V. 1984. Pepper mild mottle virus, a tobamovirus infecting pepper cultivars in Sicily. Phytopathology 74:405-410.
23. Wetter, C., Dore, I., and Bernard, M. 1987. Bell pepper mottle virus, a distinct tobamovirus infecting pepper. J. Phytopathol. 119:333-344.