Effects of Temperature on Disease Development of Tomato Mosaic Virus in *Capsicum annuum* in Hydroponic Systems

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ABSTRACT

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Tomato mosaic virus (ToMV) induced severe root, stem, and foliar symptoms when pepper (*Capsicum annuum* cv. Hungarian Wax) plants were root inoculated in a recirculating hydroponic system during warm summer months. Plant height, root length, root dry weight, and shoot dry weight of root-inoculated peppers were all significantly less than noninoculated control plants. When plants were root inoculated in cooler winter months, plant symptoms were less severe, and only root lengths of inoculated plants were shorter than those of noninoculated control plants. The numbers of local lesions observed on *Chenopodium amaranticolor* leaves inoculated with nutrient solution or hydroponic filter-sludge samples increased between 28 and 35 days after peppers were root inoculated in a recirculating hydroponic system, regardless of season. The increase in the numbers of local lesions on *C. amaranticolor* coincided with the occurrence of root symptoms on root-inoculated peppers and is believed to indicate the release of viable ToMV inoculum from infected plant roots. In growth chamber experiments, the effects of temperature on root- or foliar-inoculated peppers were similar; the greatest difference between inoculated and noninoculated plants occurred at 24°C. Moderate or severe symptoms failed to develop on either root- or foliar-inoculated plants incubated at 18°C but were observed on foliar-inoculated plants incubated at 32°C. The severity of foliar systemic symptoms and the rate of disease development were greatest in foliar-inoculated pepper plants incubated at 24°C.

Additional keywords: aeroponic, greenhouse, seasonal effects

Severe outbreaks of tomato mosaic virus (ToMV) occurred in 1985 (18) and 1993 on hydroponically grown peppers (*Capsicum annuum* L. cv. Hungarian Wax) in greenhouses at The Land, Epcot, Lake Buena Vista, Fla. Initial symptoms included chlorosis of terminal and mid-canopy leaves followed by stem necrosis and leaf abscission. Plant death occurred in young plants (5 to 8 weeks old) within 14 to 21 days after infection in the pepper cultivar Hungarian Wax but was delayed or inhibited in resistant cultivars (18). Although initial sources of inoculum were not identified, ToMV probably was introduced into the greenhouses via mechanical transmission from greenhouse workers or visitors. ToMV can persist on greenhouse structures (7), workers' clothing (7,8), and horticultural tools (3). Pepper seeds were not suspected as primary sources of inoculum because seed assays were negative for ToMV and the same seedlots were used for several crops before and after the disease outbreaks without producing symptomatic plants. Disease control in both outbreaks was achieved by removing symptomatic plants and sanitizing all hydroponic plant production systems, horticultural tools, and greenhouse structures with 0.26% sodium hypochlorite solutions.

Secondary spread of ToMV was believed to be due to mechanical transmission via contaminated horticultural tools and workers' hands. However, during the 1985 ToMV outbreak contaminated nutrient solution in a recirculating hydroponic system was suspected as an additional source of secondary inoculum (18). Virus transmission via naturally contaminated or artificially inoculated nutrient solution has been demonstrated for cucumber green mottle mosaic virus (17), lettuce big vein agent (17), melon necrotic spot virus (1), potato X virus (20), tobacco mosaic virus (19,20,25), tobacco necrosis virus (17,25), tomato bushy stunt virus (20), and tomato mosaic virus (17). Development of foliar symptoms on root-inoculated plants was generally slow, often taking between 2 and 5 months to occur (17,19,20). Furthermore, seasonal effects on symptom development on root-inoculated plants were described for tobacco mosaic virus; symptoms developed more rapidly in warmer summer months than in cooler winter months (19,20). In preliminary tests of the current study, we observed rapid development (2 to 4 weeks) of severe root and foliar symptoms with ToMV on root-inoculated peppers during the summer (A. C. Schuerger and W. Hammer, *unpublished*). The objectives of this study were to determine the effects of temperature on ToMV symptom development in root- or foliar-inoculated peppers and to determine seasonal effects on symptom development in root-inoculated peppers grown in a recirculating hydroponic system.

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MATERIALS AND METHODS

Inoculum preparation. Inoculum of tomato mosaic virus (ToMV) (Epcot isolate described previously) (18) was prepared by homogenizing 240 to 280 g (fresh weight) of symptomatic leaves from systemically infected peppers (*C. annuum* cv. Hungarian Wax) in a blender with 1,400 ml of 0.2 M phosphate buffer (pH 7). Extracts were filtered through two layers of cheesecloth and added directly to the nutrient solution in the greenhouse and growth chamber experiments in which plants were root inoculated. Control plants were treated with plant extracts prepared in phosphate buffer with leaves from healthy and asymptomatic pepper plants. For foliar-inoculated pepper plants, inoculum of ToMV was prepared by triturating 10 to 12 symptomatic pepper leaves in 30 ml of 0.2 M phosphate buffer (pH 7). Two to three leaves per plant were dusted with 320-grit Carborundum and inoculum rubbed onto leaves with sterile cheesecloth.

Greenhouse experiment. Four aeroponic tanks (152 × 81 × 122 cm) (Fig. 1) were constructed of reinforced fiberglass with interior walls coated with a smooth isothalic gelcoat finish (Cook Composites, Orlando, Fla.). Exterior walls were covered with 5 cm of a thermal insulation material (Trymer 9501 rigid foam, Dow Chemical Co., Midland, Mich.) and spray painted with white acrylic latex enamel to reduce temperature fluctuations within the tanks. White expanded polystyrene foam boards (Dyplast Co., Lakeland, Fla.) were placed into recessed grooves at the tops of each tank (Fig. 1) and functioned as primary plant supports. Plants were irrigated with recirculating mist systems (Fig. 1A) constructed of 2.54-cm PVC pipe (schedule 40). Each misting system consisted of six stainless steel spray nozzles (80 degree fan pattern, W. A. Westgate Co., Inc., Davis, Calif.) spaced 38 cm apart on a common manifold and mounted on risers 15 cm above the bottom of the tank. Each mist system was operated by a self-priming centrifugal epoxy pump (model 17020-0001, Jabsco Products ITT, Costa Mesa, Calif.) and 1/4 HP motor. A closed-loop recirculating cooling system was constructed of 1.27-cm PVC pipe (sche-

dule 120) to reduce the temperature of the nutrient solution (Fig. 1B). Chilled water from a greenhouse evaporative cooling system (CELDEK system, Munters, Inc., Fort Myers, Fla.) was pumped through the PVC pipes to each tank. Pressure was regulated at each tank (Fig. 1B) to increase the uniformity of temperature control among the four tanks. Temperatures within the aeroponic tanks fluctuated between 26 and 30°C when the cooling system was operated and between 30 and 34°C when the cooling system was not operated during preliminary tests.

Pepper seeds were germinated in 2.5-cm rockwool cubes (Grodania A\S, Hedehusene, Denmark), and then eight seedlings were transplanted into each aeroponic tank 26 and 30 days after sowing. Pepper seedlings were spaced 38 cm apart in double rows and inserted through polystyrene foam boards. A sterilized chicken-wire cage was placed around each pepper plant to prevent leaf contact when plants increased in size during the experiment.

Each aeroponic tank contained 125 liters of nutrient solution (21), and roots were misted continuously throughout the experiment. The hydrogen ion concentration of the nutrient solution was maintained at pH 6 ± 0.2 with a digital pH controller (model 7142, Cole-Parmer Instrument Co., Chicago, Ill.) and 0.6 N H_2SO_4.

Ten days after transplanting, roots of four pepper plants in each tank were wounded by removing 1 and 2 cm of tissue from the bottom of each root system. Four plants per tank were not wounded. Pepper plants in two randomly selected aeroponic tanks were inoculated by adding 700 ml of the ToMV inoculum described above to each tank. Inoculated and noninoculated plants were maintained for an additional 60 days. Nutrient solution samples containing plant debris and sludge from 100-mesh polypropylene in-line filters (Fig. 1A) were obtained every 7 days from each of the aeroponic tank systems. The nutrient solution samples and filter-sludge samples were rubbed separately onto leaves of *Chenopodium amaranticolor* Coste & Reyn. previously dusted with 320-grit Carborundum. Local lesions for each sample were counted 14 days later.

Sixty days after plant inoculation, symptoms (foliar mosaic, leaf chlorosis or necrosis, leaf drop, stem and fruit necrosis, plant wilt, and root necrosis), plant heights, root lengths, and shoot and root dry weights were recorded for each pepper plant. In addition, randomly selected leaves, stem sections, and root pieces were taken from each inoculated and noninoculated plant, triturated in separate volumes of 30 ml of phosphate buffer (pH 7), and rubbed onto individual leaves of *C. amaranticolor*. The presence or absence of local lesions on individually inoculated leaves of *C. amaranticolor* was recorded after 14 days.

Relative humidity and air temperature (Table 1), and photosynthetically active radiation (PAR), were automatically collected by a Campbell Scientific CR7X Measurement and Control System (Campbell Scientific, Inc., Logan, Utah). The experiment was conducted twice in warm-season months (8 June to 1 November 1990) and twice in cool-season months (27 November 1990 to 25 April 1991).

Growth chamber experiments. In the first of two plant growth chamber experiments, 16 pepper seedlings (per growth chamber) were grown in 2.5-cm rockwool cubes for 21 days and then transplanted into an organic soil mix (Speedling Peat-Lite Mix, Speedling Inc., Sun City, Fla.) in 6-inch pots. Plants were transferred to

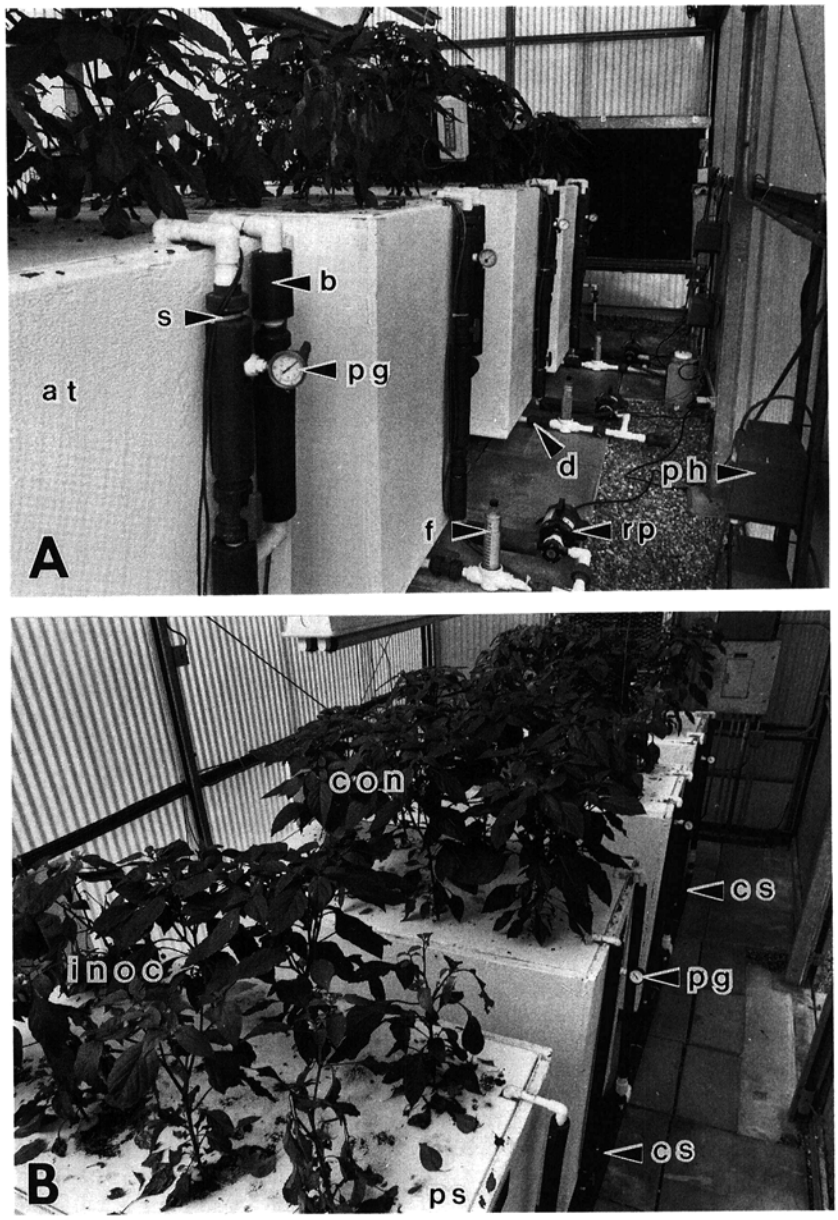

Fig. 1. Aeroponic tanks (a t) used for long-term greenhouse experiments. **(A)** Each aeroponic tank was equiped with a separate recirculating nutrient delivery system. The nutrient delivery system was constructed of 2.54-cm PVC pipe (schedule 40) in which nutrient solution was pumped via a drain line (d) through a 100-mesh polypropylene filter (f) and a 1/4 HP recirculation pump (r p). Nutrient solution was returned to the aeroponic tank via a supply line (s) in which line pressure was monitored with a gauge (p g) and maintained at 0.85 km per cm^2 (12 lb/in^2) by adjusting the back-pressure of nutrient solution in a bypass line (b). Six stainless-steel spray nozzles were connected to a common manifold at the terminal end of the supply line. The hydrogen ion concentration was maintained at pH 6 ± 0.2 with a remote controller (p h). **(B)** A closed-loop recirculating cooling system (c s) was installed on the side of the aeroponic tanks opposite the recirculating nutrient delievery system. The aeroponic tank cooling system pumped water from a greenhouse evaporative cooling system (not shown) through 1.27-cm PVC pipe (schedule 120). Pressure and flow were regulated with the aid of pressure gauges (p g) to maintain uniformity of temperature control among the four aeroponic tanks. (i n o c = inoculated plants; c o n = noninoculated control plants; p s = polystyrene foam board.)

Table 1. Effects of season on the development of symptoms of tomato mosaic virus (ToMV) in peppers grown in aeroponic tanks in a greenhouse

Experiment no.	Calendar (1990 to 1991)	Mean ambient experimental temperature (C)/relative humidity(%)	Mean ambient day/night temperature (C) during inoculation[y]	Days to first root symptoms	Plants with root necrosis (%)[z]	Plant wilt (%)[z]
ToMV-1	June to August	27.9/80.4	31.0/25.0	14	100	50
ToMV-2	September to November	27.0/68.9	32.9/25.8	16	100	56
ToMV-3	November to January	26.0/51.5	25.4/21.8	30	44	19
ToMV-4	February to April	24.9/55.9	25.9/22.7	30	50	6

[y] Temperature measured 14 days after ToMV was added to the aeroponic tanks.
[z] Root necrosis and plant wilt were rated at the termination of the experiments (60 days after plant inoculation) ($n = 16$).

Fig. 2. Pepper plant (*Capsicum annuum* cv. Hungarian Wax) with severe foliar symptoms caused by tomato mosaic virus (ToMV) in a root inoculated greenhouse experiment utilizing an aeroponic nutrient delivery system. Photograph was taken at harvest (60 days after root-inoculation) during a warm-season experiment; foliar and stem necrosis occurred between 28 and 35 days after root inoculation.

Table 2. Effects of tomato mosaic virus (ToMV) on growth of *Capsicum annuum* plants that were root inoculated in an aeroponic system

Plant characteristic	Noninoculated plants		Inoculated plants	
	Not wounded	Wounded	Not wounded	Wounded
June to November 1990 (warm season)				
Plant height (cm)	115.2 a[z]	115.7 a	73.1 c	87.9 b
Root length (cm)	123.8 a	124.0 a	66.8 b	72.4 b
Shoot dry weight (g)	83.5 a	86.9 a	39.1 b	48.1 b
Root dry weight (g)	5.9 a	5.7 a	2.4 b	2.7 b
November 1990 to April 1991 (cool season)				
Plant height (cm)	102.6 a	103.4 a	96.6 a	97.7 a
Root length (cm)	132.3 a	135.3 a	107.4 b	109.9 b
Shoot dry weight (g)	75.5 a	67.3 a	60.0 a	70.9 a
Root dry weight (g)	5.1 a	5.2 a	4.4 a	4.4 a

[z] Treatments in rows followed by the same letter were not significantly different based on analysis of variance and protected least-squares mean separation tests ($P > 0.05$; $n = 16$).

growth chambers maintained at 18, 24, or 32°C under a 12-h photoperiod. Light intensity and vapor pressure deficit were maintained for each temperature at 250 to 275 µmol m^{-2} s^{-1} and 10.7 mmHg, respectively. Plants were watered daily with a nutrient solution adjusted to pH 6 (21). Eight randomly selected plants in each growth chamber were inoculated 14 days after transplanting. The two oldest true leaves of each inoculated plant were rubbed with inoculum containing ToMV prepared from systemically infected Hungarian Wax pepper leaves. After 14 days, symptoms (foliar mosaic, leaf chlorosis or necrosis, stem necrosis, leaf drop, plant wilt) and plant heights were recorded for each plant. Pepper plants were then transferred to greenhouse benches and maintained for 7 days to determine if symptoms would develop on asymptomatic plants. The experiment was conducted four times.

In a second plant growth chamber experiment, 10 pepper seedlings (per growth chamber) were grown for 21 days in 2.5-cm rockwool cubes in a greenhouse and then transplanted into 3.8-liter opaque plastic vessels containing 3.6 liters of nutrient solution (pH 6). Plants were transferred into growth chambers maintained at 18, 24, or 32°C under a 12-h photoperiod. Light intensity and vapor pressure deficit were maintained for each temperature at 250 to 275 µmol m^{-2} s^{-1} and 10.7 mmHg, respectively. Nutrient solution was added daily to each container to maintain a volume of 3.6 liters. The pH was adjusted daily with 0.03 N HNO_3. After 14 days, five randomly selected peppers were root inoculated by adding 50 ml of ToMV inoculum prepared in phosphate buffer to the nutrient solution of each opaque plastic vessel. Control plants were treated with plant extracts prepared in phosphate buffer, as described above. After 35 days, symptoms (stem necrosis, leaf drop, plant wilt, root necrosis), root and shoot dry weights, and plant heights were recorded. The experiment was conducted three times.

Statistical procedures. Statistical analyses were conducted with a PC-based Statistical Analysis System (SAS Institute, Inc., Cary, N.C.). Data from greenhouse experiments were analyzed with analysis of variance (ANOVA) and protected least-squares mean separation tests ($P \leq 0.05$). Data from plant growth chamber tests were analyzed by regression with the PROC RSREG procedure in SAS ($P \leq 0.05$). Error bars in figures on the effects of temperature on disease represent 95% confidence intervals (CI) calculated with the PROC GLM procedure in SAS. Only nonoverlapping 95% CIs were considered significantly different at specific temperatures ($P \leq 0.05$). Measured responses were similar among separate repetitions of individual experiments.

RESULTS

Greenhouse experiment. Small necrotic flecks were first observed on pepper roots approximately 2 weeks after plants were inoculated in aeroponic tanks during warmer months and approximately 4 weeks after plants were inoculated during cooler months of the year (Table 1). As symptoms intensified, necrotic flecks coalesced into larger lesions, and eventually necrosis extended to most of the inoculated pepper roots. Foliar symptoms were first observed 2 to 3 weeks after root symptoms were observed, regardless of season. Severe foliar symptoms included leaf veinal necrosis, leaf abscission, and necrosis and collapse of stem tissues (Fig. 2). The severity and incidence of root necrosis, and the percentage of inoculated plants that wilted during the experiments, were higher when plants were inoculated during warmer months than when plants were inoculated during cooler months of the year (Table 1). Symptom development in root-inoculated peppers was positively correlated to temperature and relative humidity (Table 1) but was not affected by light intensity (PAR) (data not shown).

Plant heights, root lengths, shoot dry weights, and root dry weights of inoculated peppers were significantly less than those of noninoculated control plants dur-

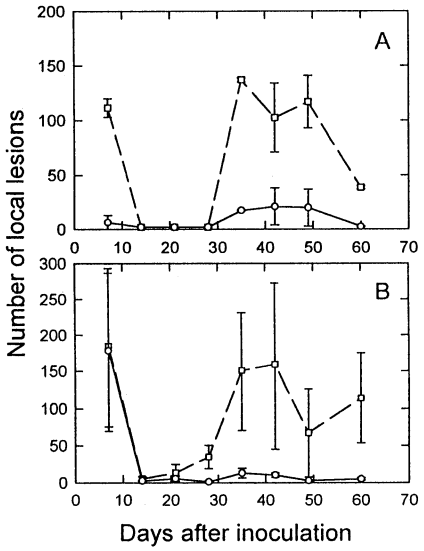

Fig. 3. Numbers of local lesions observed on *Chenopodium amaranticolor* leaves inoculated with nutrient solution (-O-) or filter-sludge (-□-) samples from **(A)** warm-season or **(B)** cool-season experiments. Error bars represent standard errors ($n = 4$).

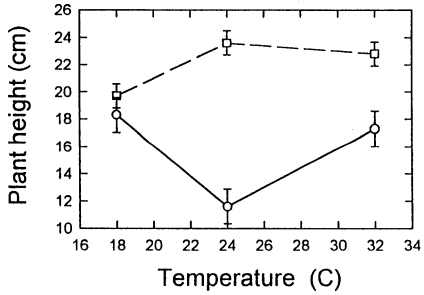

Fig. 4. Effects of temperature on plant growth of foliar-inoculated pepper plants (*Capsicum annuum* cv. Hungarian Wax) maintained in plant growth chambers. Plant heights of noninoculated control (-□-) and foliar-inoculated (-O-) plants were similar at 18°C but were significantly different at 24 and 32 C ($P \leq 0.05$, $n = 32$). Error bars represent 95% confidence intervals (CI) for the predicted regression lines at specific temperatures; only nonoverlapping 95% CIs were considered significantly different. Effects of temperature on plant height were described best by second-order polynomials: noninoculated plants ($Y = -15.674 + 2.939(T) - 0.054(T^2)$; $r^2 = 0.319$) and foliar-inoculated plants ($Y = 94.929 - 6.612(T) + 0.131(T^2)$; $r^2 = 0.409$); in which Y is the predicted value and T is the temperature.

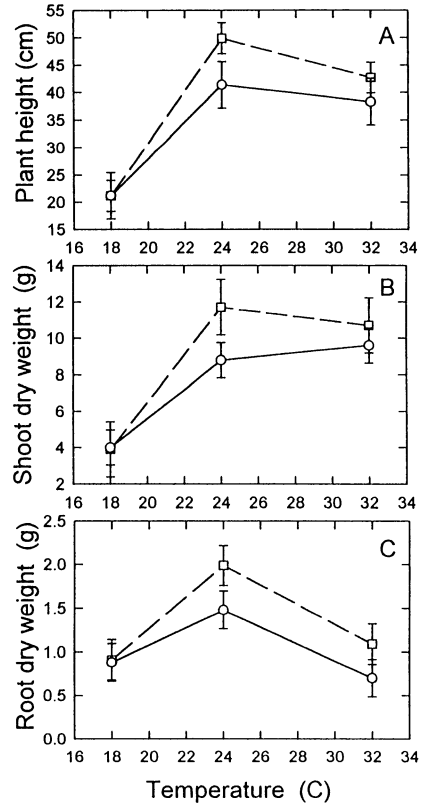

Fig. 5. Effects of temperature on plant growth of root-inoculated pepper plants (*Capsicum annuum* cv. Hungarian Wax) maintained in plant growth chambers. **(A)** Plant heights, **(B)** shoot dry weights, and **(C)** root dry weights of noninoculated control (-□-) and root-inoculated (-O-) plants were similar at 18 and 32°C but were significantly different at 24°C ($P \leq 0.05$, $n = 15$). Error bars represent 95% confidence intervals (CI) for the predicted regression lines at specific temperatures; only nonoverlapping 95% CIs were considered significantly different. Effects of temperature on plant height were described best by second-order polynomials: noninoculated plants ($Y = -239.743 + 21.786(T) - 0.405(T^2)$; $r^2 = 0.844$) and root-inoculated plants ($Y = -155.114 + 14.617(T) - 0.268(T^2)$; $r^2 = 0.559$). Effects of temperature on shoot dry weight were described best by second-order polynomials: noninoculated plants ($Y = -64.322 + 5.645(T) - 0.103(T^2)$; $r^2 = 0.606$) and root-inoculated plants ($Y = -32.096 + 2.910(T) - 0.050(T^2)$; $r^2 = 0.655$). Effects of temperature on root dry weight were described best by second-order polynomials: noninoculated plants ($Y = -11.380 + 1.059(T) - 0.021(T^2)$; $r^2 = 0.549$) and root-inoculated plants ($Y = -7.089 + 0.699(T) - 0.014(T^2)$; $r^2 = 0.417$). (Y is the predicted value, and T is the temperature.)

ing warm-season experiments (Table 2). In contrast, only root lengths of inoculated plants were significantly shorter than those of noninoculated plants during cool-season experiments. Root wounding did not affect the growth of noninoculated control plants in either the warm- or cool-season experiments; nor did root wounding affect the severity of viral symptoms, except that the heights of inoculated and wounded peppers were greater than those of inoculated and not wounded peppers during warm-season experiments (Table 2). The level of root necrosis was unaffected by root wounding.

The numbers of local lesions observed on *C. amaranticolor* leaves inoculated with nutrient solution or filter-sludge samples were generally high at 7 days after inoculation (up to 130 to 160 per leaf), dropped to near zero between 14 and 21 days after inoculation (5 to 20 per leaf), and increased dramatically between 28 and 35 days after inoculation (up to 120 to 150 per leaf) (Fig. 3). The increase in the numbers of local lesions between 28 and 35 days coincided with the first occurrence and subsequent intensification of root symptoms on inoculated plants. The numbers of local lesions on *C. amaranticolor* leaves inoculated with nutrient solution or filter-sludge samples from ToMV-treated aeroponic tanks were very similar between warm-season (Fig. 3A) and cool-season (Fig. 3B) experiments. Local lesions were not observed on *C. amaranticolor* leaves treated with samples from the noninoculated control plants, indicating that cross-contamination did not occur between inoculated and noninoculated aeroponic systems (data not shown).

At harvest, randomly selected samples from symptomatic leaf, stem, and root tissues from inoculated pepper plants were tested for the presence of ToMV by rubbing samples onto leaves of *C. amaranticolor*. The presence of local lesions was observed in 82% of symptomatic root samples (46 of 56 samples), 54% of symptomatic stem samples (14 of 26 samples), and 2% of symptomatic leaf samples (1 of 58 samples). Results were generally consistent between warm- and cool-season experiments.

Growth chamber experiments. Foliar-inoculated pepper plants maintained at 24 and 32°C for 14 days in plant growth chambers were significantly smaller than noninoculated control plants (Fig. 4). The greatest difference between inoculated and noninoculated peppers occurred when plants were incubated at 24°C. Foliar-inoculated peppers were not different from noninoculated peppers when plants were incubated at 18°C. Severe foliar symptoms (foliar mosaic and chlorosis, leaf and stem necrosis, leaf abscission, and plant wilt) developed on 3, 100, and 69% of plants ($n = 32$) inoculated at 18, 24, and 32°C, respectively. When asymptomatic plants maintained at 18 and 32°C (97 and 31%, respectively) were transferred to greenhouse benches, 30 of 31 plants (96% of asymptomatic plants) originally maintained at 18°C wilted after 7 days, but no change was observed in the percentage of asymptomatic plants originally maintained at 32°C. Typically, foliar-inoculated pepper plants maintained in greenhouses (24 to 28°C) wilted between 12 and 17 days (A. C. Schuerger, *unpublished*).

In a second series of growth chamber studies in which plants were root-inoculated and incubated at 18, 24, or 32°C, symptom development was less severe compared with that of foliar-inoculated plants incubated at the same temperatures. Visual symptoms on root-inoculated peppers incubated at 24 or 32°C generally included root necrosis and small necrotic lesions (2 to 3 cm in length) on lower stems (80 and 90%, respectively), and rarely included wilt or abscission of lower leaves (less than 10% of inoculated plants at 24 or 32°C) ($n = 15$). No symptoms were observed on root-inoculated peppers

incubated at 18°C. Plant heights, shoot dry weights, and root dry weights of root-inoculated peppers were significantly smaller than those of noninoculated peppers when plants were incubated at 24°C but not different when plants were incubated at 18 or 32°C (Fig. 5).

DISCUSSION

Seasonal effects on symptom expression in ToMV-inoculated peppers were similar to the effects of temperature on disease development. During warm summer months, mean ambient temperatures were 27 to 28°C but were often as high as 32 to 35°C during the day (data not shown). In plant growth chamber experiments, temperatures of 24 or 32°C were conducive to the development of ToMV systemic symptoms in foliar-inoculated peppers, and 24°C was conducive to symptom development in root-inoculated peppers. During cool winter months, mean ambient temperatures were 25 to 26°C but were often as low as 17 to 19°C at night (data not shown). In growth chamber experiments, systemic ToMV symptoms generally did not develop in root-inoculated (0%) or foliar-inoculated (3%) peppers when plants were incubated at 18°C. These results are consistent with previous reports on the effects of temperature on symptom development with tobacco mosaic virus (TMV) and ToMV (9,11,13,16). Generally, as temperature drops below 20 to 24°C, symptom development by ToMV or TMV tends to progress slowly and the severity of symptoms decreases.

Seasonal effects on symptom development in root-inoculated tomato plants in hydroponic nutrient solutions have been described for tobacco mosaic virus (19, 20), but no correlation of seasonal effects to particular environmental factors were described. Effects of cool temperatures (≤20°C) on viral symptom expression may explain reports of long delays (up to 5 months) in development of viral symptoms reported in root-inoculated plants in northern European greenhouses (17,19,20). Mean daily temperatures in northern European greenhouses are often 17 to 20°C (Bent Vestergard, *personal communications*). In our studies, symptom development during warm summer months was very rapid, being on the order of 2 to 3 weeks, instead of the 2 to 5 months reported in cooler climates (17,19,20). Although symptom development was positively correlated with temperature in the ToMV and Hungarian Wax pepper pathosystem, possible effects of other environmental parameters also should be considered. In the current study, humidity was positively correlated to the rate of ToMV symptom development, but no tests were conducted to establish a cause and effect relationship between humidity and symptom development. However, Foster and Ross (11) found that cell-to-cell movement of tobacco mosaic virus in tobacco plants increased as air temperature increased (20 to 30°C) but was unaffected by humidity, leaf age, day length, or light intensity. An effect of light intensity (PAR) was not observed in the current study on ToMV root-inoculated peppers in an aeroponic system.

Plants can become infected with TMV at low temperatures (≤20°C) but fail to develop symptoms unless moved to higher conducive temperatures under which symptom development is unusually rapid (9,10). In the current study, ToMV foliar-inoculated peppers incubated at 18°C generally failed to exhibit systemic symptoms until moved to higher temperatures (24 to 26°C) in a greenhouse. In a greenhouse system, plants may become infected at lower temperatures during short periods of cool weather that are not conducive to symptom development, but when environmental conditions change to permissive temperatures rapid and severe symptoms may develop.

Based on the results of the current study and on the reviewed literature, an integrated pest management (IPM) program for managing ToMV in recirculating hydroponic systems is proposed. First, sterilization of fresh water supplies likely will be required if surface waters are used for mixing nutrient solutions. Many plant viruses have been reported as contaminants in river, canal, and lake waters (14,15,23, 24). We found no reports in the literature that detected plant viruses in ground water supplies, and ToMV was not detected in the ground water supply used at The Land when tested in 1985 and 1993 (A. C. Schuerger, *unpublished*). Second, other primary sources of inoculum besides contaminated water must be controlled through sanitation and quarantine procedures. Other primary sources of tobamoviruses include contaminated seed (6,8), plant transplants (4), contaminated root debris (8), workers' hands and clothing (7, 8), horticultural tools (3), birds (5), and possibly smoking tobacco (2). Third, if a disease outbreak occurs, infected plants should be rogued with subsequent sanitation of horticultural equipment and greenhouse structures. Pategas et. al. (18) reported successful eradication of ToMV in a greenhouse hydroponic system by repeatedly sanitizing horticultural tools, ladders, greenhouse structures, and workers' clothing. Fourth, recirculating nutrient delivery systems should be sterilized to prevent recontamination of disease-free transplants. Recirculating nutrient delivery systems likely become infested with virulent virions when infected root materials such as root-cap cells, plasmolized root hairs, epidermal and cortical fragments, or broken root branches, are sloughed off diseased and moribund root systems. In the current study, observed fluctuations in the numbers of local lesions on *C. amaranticolor* leaves inoculated with filter-sludge samples from ToMV-infested aeroponic systems support the conclusion that virulent propagules of ToMV were released from infected roots after root symptoms were observed. Release of viral inoculum from infected roots has been reported for tobacco mosaic virus (12,20,25) and tobacco necrosis virus (22,25). We believe that it is insufficient to limit plant removal to only symptomatic plants in recirculating hydroponic systems because virulent virions may be released into nutrient solutions prior to plant removal, and, thus, the virions may be transported to other sections of the recirculating systems. Rogueing only symptomatic plants may be a viable component of an IPM program if the crop is near maturity and subsequent secondary spread of the pathogen is not a major concern, but eradication of the viral pathogen is unlikely until the entire recirculating hydroponic system is sanitized. Fifth, the low detection of ToMV on *C. amaranticolor* from samples of symptomatic leaves of root-inoculated plants in the aeroponic greenhouse study supports the conclusion that foliar symptoms in ToMV root-inoculated pepper plants may not be due to the presence of the pathogen, but rather may be a host response to severe root and stem tissue damage. Similar foliar symptoms on foliar-inoculated plants always yielded positive results in local lesion assays or host inoculation tests (A. C. Schuerger, *unpublished*). The importance of this conclusion, if verified with further root-inoculated versus foliar-inoculated tests with other plant viruses, is that sampling of symptomatic foliar tissues may be negative for some viral pathogens if plants are infected via roots. Broadbent (4) showed that systemic movement of TMV from root-inoculated tissues into the upper canopy of tomato was highly variable, often requiring between 3 weeks and 6 months for the appearance of visible symptoms. If a plant cultivar is highly sensitive to a viral pathogen such that foliar wilt occurs more rapidly than the virus can progress up into foliage, then wilted leaves may be free of viable propagules. If a viral pathogen is suspected as the causal agent of plant disease in a hydroponic system, all types of symptomatic tissues should be tested separately for the presence of the pathogen.

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