Evaluation of Eggplant Rootstocks and Pepper Varieties for Potential Resistance to Isolates of *Phytophthora capsici* from Michigan and New York

J. M. Foster, former Graduate Research Assistant, R. P. Naegele, Graduate Research Assistant, and M. K. Hausbeck, Professor, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing 48824

Abstract

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Phytophthora capsici is a soilborne pathogen of major economic importance in pepper, and of less importance in tomato and eggplant production. As soil fumigation becomes more expensive and limited, and fungicide insensitivity of *P. capsici* isolates becomes more prevalent, grafting is quickly becoming an industry-favored method to control soilborne diseases. Greenhouse experiments were performed to evaluate an eggplant cultivar (Classic), two eggplant lines (EG195, EG203), a pepper line (CM334), and three pepper cultivars (Paladin, Camelot, and Red Knight) for root rot resistance to 14 *P. capsici* isolates. The isolates showed various degrees of virulence between pepper and eggplant in both experiments. Both eggplant and one pepper lines showed moderate resistance to the most virulent isolates tested in ex-

periment one. The partially resistant pepper cultivar, Paladin, was significantly more susceptible than CM334 and the eggplant lines, but was still resistant to most isolates. In the second experiment, the eggplant cultivar Classic and the susceptible pepper cultivar Red Knight were both susceptible to most isolates tested, while EG203 and EG195 were resistant to most isolates. The two eggplant breeding lines, EG195 and EG203, showed moderate resistance to all isolates tested in both experiments. This is the first reported evaluation of eggplant resistance to *P. capsici*. Further research is warranted to test eggplant lines EG195 and EG203 for resistance to a wide range of soilborne pests and to evaluate their usefulness as *P. capsici*-resistant rootstocks for peppers, tomatoes, and eggplants.

In 2011, Michigan produced over 10,000 ha of vegetables susceptible to the pathogen *Phytophthora capsici* Leonian, including species in the Cucurbitaceae, Fabaceae, and Solanaceae (1). Pepper, tomato, and eggplant, the three major cultivated solanaceous species, are susceptible to *P. capsici* (10,18). Currently, no pepper or tomato cultivar has demonstrated resistance to all Michigan isolates tested (5,11,20,22). In Michigan, pepper (*Capiscum annuum* L.) production accounts for over \$12 million in farm revenue, with *Phytophthora* crown and root rot being a major limitation. On eggplant (*Solanum melongena* L.), the most common symptom of *P. capsici* infection is fruit rot, and although crown and root rot can occur, it is far less common (7). In a field study, eggplant had lower area under the disease progress curve (AUDPC) values for *Phytophthora* crown and root rot than all other susceptible vegetables studied, including pepper, zucchini, pumpkin, and summer squash (11). Currently, control of *P. capsici* in susceptible crops is predominantly achieved through chemical and cultural practices such as preplant soil fumigation, raised plant beds, mulching to reduce soil splash, using well water for irrigation, and frequent fungicide applications (10,26).

Genetic resistance is needed for long-term, sustainable disease control. Breeding for genetic resistance can take years, but grafting can provide an alternative to rapidly utilize genetic resistance. Grafting susceptible scions to resistant rootstocks is one method to limit *Phytophthora* root rot. Grafted plants are used extensively in Europe and Asia for control of *Verticillium* wilt, nematodes, and other soilborne diseases of tomato and eggplant (3,4,19,20). Besides providing disease resistance, rootstocks that are more vigorous have the potential to increase yields by increasing nutrient absorption, heat tolerance, and fruit quality (14). It is estimated that grafted plants account for up to 40% of the total production in

some Asian countries, while in the United States, 70% of greenhouse production uses grafted tomato (17). Pepper cultivars, already tested for resistance to *P. capsici*, have shown varying degrees of grafting compatibility to susceptible pepper scions, and limited compatibility with tomato and eggplant (2,12,23,28,29). A pepper cultivar from Italy, Grafito, showed good resistance when used as a rootstock for control of *Phytophthora* root rot and *Verticillium* wilt in pepper, and no reductions in yield (20). Criollo de Morelos (CM334), a serrano-type landrace pepper with high levels of resistance to *P. capsici*, used as a rootstock, reduced overall disease incidence in the field, but in noninfested soils, pepper yields were significantly lower than for the nongrafted controls (6,20).

In North America, it is estimated that 40 million grafted plants are used annually in greenhouse production of tomato to control a number of diseases (15). Field use of grafted plants is expensive and limited, and most rootstocks are not well adapted to field growth. Eggplant is more tolerant to *Phytophthora* root rot than pepper or tomato, making it a likely choice for *P. capsici* resistant rootstock (7,13). The eggplant lines EG195 and EG203 are resistant to bacterial wilt, root-knot nematode, and tomato *Fusarium* wilt, and have been used in the greenhouse as rootstocks for tomato (3,24).

The objectives of this research were: (i) to evaluate two breeding lines of eggplant used for grafting tomatoes for resistance to *Phytophthora* root and crown rot, and (ii) to compare the level of resistance among potential eggplant rootstocks and pepper rootstocks against *P. capsici* isolates from two different states.

Materials and Methods

Isolate selection and inoculum preparation. Fourteen isolates were selected from the long-term culture collections of Mary Hausbeck, Michigan State University (MSU), and Christine Smart, Cornell University. The isolates were classified according to mating type (A1 or A2), sensitivity to mefenoxam (S = sensitive and I = insensitive), and host plant (16). Isolates OP97 (A1, S, pickling cucumber), SP98 (A2, S, pumpkin), 12889 (A1, I, pepper), and SFF3 (A2, I, pickling cucumber) were from Michigan, and isolates 13351 through 13360 (A1, S, eggplant) were from New York.

Corresponding author: M. K. Hausbeck, E-mail: hausbec1@msu.edu

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P. capsici isolates were maintained on unclarified V8 agar (UCV8, 16 g agar, 30 mM CaCO₃, 160 ml unfiltered V8 juice, and 840 ml distilled water) and grown under constant fluorescent light at room temperature (21 ± 2°C) for 7 days. For the root and crown rot inoculation, millet seed medium was prepared according to the method of Quesada-Ocampo and Hausbeck (22) and used to prepare each isolate for inoculum.

Root and crown rot screens. Two experiments were conducted twice to evaluate pepper (*C. annuum*) and eggplant (*S. melongena* L. var. *esculentum*) seedlings for susceptibility to *Phytophthora* root and crown rot in a polyethylene greenhouse at MSU's Horticulture Teaching and Research Center, East Lansing, MI. In the first experiment, four *P. capsici* isolates (OP97, SP98, SFF3, and 12889) were tested on eight plants each of three pepper cultivars, and one pepper and two eggplant lines, and the experiment was repeated one time (Table 1). A second experiment tested 11 *P. capsici* isolates (12889, 13351 to 13360) against eight plants each of one pepper and one eggplant cultivar and two eggplant lines, and this experiment was repeated once (Table 1). For both experiments, commercial eggplant and pepper seeds were purchased from Seedway, LLC (Hall, NY). Experimental eggplant lines were developed at AVRDC-The World Vegetable Center, and seed for both the experimental eggplant and pepper lines were provided by Richard Hassel (eggplant) (Clemson University) and George Moriarty (pepper) (Cornell University). Seeds were sown into 72-cell flats containing potting media (BACCTO Professional Planting Mix, Michigan Peat Company, Houston, TX) and grown in a greenhouse with a 14-h photoperiod. Transplants were grown until all peppers and eggplants developed three to four and two to three true leaves, respectively.

Pepper and eggplant seedlings were transplanted into individual 1.5-liter pots filled with potting media. Pots were arranged in a complete randomized design on raised benches in a greenhouse and spaced to exclude pathogen splash dispersal. Eight plants were used for all isolates and also for including a negative control consisting of sterile noninoculated millet seed. Each plant was considered a replicate. Each pot was inoculated with 1 g of millet seed inoculum placed into the potting media 2.5 cm below the surface, adjacent to the pepper or eggplant's root mass, 24 h after transplanting. Each plant was individually irrigated every 1 to 2 days and fertilized three times a week with 200 ppm of Peters 20-20-20 (Scott's Company, Marysville, OH). Irrigation water was amended with phosphoric acid at 132 ppm twice weekly to maintain the media pH at approximately 6.0 to 6.5. The pH of the media was checked monthly by collecting random samples and using a pH meter (Hanna Instruments, Woonsocket, RI). The average temperature in the greenhouse was 18.6°C (minimum 1.0°C, maximum 41.6°C), and the average relative humidity was 59.7% (minimum 20.7%, maximum 96.2%) during the course of both experiments.

Approximately 10% of the plants exhibiting *P. capsici* symptoms were sampled to reisolate the pathogen. The roots and crown

of each plant were washed with deionized water and sprayed with a 70% ethanol solution. Three portions each of the root and crown tissue (1 to 3 mm) adjacent to symptomatic tissue were excised and plated onto BARP (benomyl, ampicillin, rifampicin, and pentachloronitrobenzene)-amended UCV8 agar plates. Cultures were incubated at room temperature under constant fluorescent lighting for 3 days and checked microscopically (×200) to confirm *P. capsici* using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (32). Hyphal-tips of *P. capsici* cultures were transferred onto new BARP-amended UCV8 agar plates and maintained as described. After 7 days, each resulting isolate was screened for mefenoxam sensitivity and mating type according to the methods of Lamour and Hausbeck (16) to confirm that the isolate phenotype matched that of the isolate used as inoculum.

For both experiments, *Phytophthora* crown and root rot were evaluated on average every 3 days following inoculation until the plants matured and pepper fruits were 7 to 10 cm in diameter (average 73 days postinoculation). Plant health was assessed on a 0 to 4 scale (0 = healthy, 1 = minor wilting, 2 = moderate wilting, 3 = severe wilting, 4 = plant death) modified from Foster and Hausbeck (5). The area under the disease progress curve (AUDPC) was calculated according to the methods of Shaner and Finney (25) to demonstrate cumulative disease response throughout the growing period based on the assessed plant health scores.

Evaluation and data analysis. The AUDPC values were subjected to analysis of variance (ANOVA) using the PROC MIXED procedure of SAS v 9.2 (SAS Institute Inc., Cary, NC). Fisher's protected LSD was used for separation of means when effects were found to be statistically significant in ANOVA ($P \leq 0.05$). Final plant health means were calculated using PROC MEANS procedure of SAS v 9.2 ($P \leq 0.05$). The eggplant and pepper lines were considered resistant to crown and root rot if they received a mean plant health score <1, intermediately resistant if they received a final mean plant health score ≥1 but ≤2, and susceptible if they had a mean plant health score >2. Data were combined to calculate the mean plant health and AUDPC values across both replicates of the experiment unless they were significantly different ($P \leq 0.05$). Uninoculated control plants were removed from analysis to avoid violating variance assumptions of the test.

Results

Phytophthora root and crown rot screen. Susceptible pepper plants exhibited crown rot and stem lesions, wilt, and death when inoculated with *P. capsici* isolates. In both studies, stem lesions and crown rot did not develop on susceptible eggplant inoculated with *P. capsici*, but wilting occurred and was followed by plant death. Significant differences ($P \leq 0.05$) were found among AUDPC values for the isolates, pepper/eggplant lines, and the isolate × line interaction for both experiments. All *P. capsici* isolates obtained from inoculated plants had the same phenotype (mating type, mefenoxam sensitivity, and morphological features) as the isolate used for the inoculum (*data not shown*). None of the noninoculated controls showed symptoms of *P. capsici* infection.

Table 1. *Capsicum annuum* and *Solanum melongena* var. *esculentum* lines screened for root and crown rot resistance to *Phytophthora capsici* during 2008 and 2009

Line	Seed company/provider	Resistance
Experiment 1		
Camelot ^x	Monsanto Company, St. Louis, MO	Susceptible
CM334 ^y	Cornell University, Ithaca, NY	Resistant
EG195 ^z	AVRDC-the World Vegetable Center	Unknown
EG203 ^z	AVRDC-the World Vegetable Center	Unknown
Paladin ^x	Syngenta Seeds Inc., Golden Valley, MN	Intermediate
Red Knight ^x	Monsanto Company	Susceptible
Experiment 2		
Classic ^z	Harris Moran Seed Company, Modesto, CA	Unknown
EG195 ^z	AVRDC-the World Vegetable Center	Unknown
EG203 ^z	AVRDC-the World Vegetable Center	Unknown
Red Knight ^x	Monsanto Company	Susceptible

^x Bell pepper.

^y Serrano pepper.

^z Eggplant.

Table 2. Average area under the disease progress curve (AUDPC) values for *Phytophthora capsici* isolates from Michigan causing crown and root rot symptoms on eggplant and pepper (experiment 1)^y

Isolate	AUDPC ^z	Mean plant health
SP98	1 c	0.0
SFF3	6 c	0.0
OP97	42 b	1.0
12889	93 a	2.0

^y This experiment included two eggplant lines (EG195 and EG203) and four pepper lines or cultivars (Camelot, CM334, Paladin, and Red Knight).

^z AUDPC values within the same experiment with letters in common are not statistically different according to Fisher's LSD ($P = 0.05$).

In the first experiment containing four isolates, isolate 12889 caused the greatest wilting followed by isolates OP97, SP98, and SFF3 for peppers (Table 2). The eggplant lines, EG195 and EG203, were moderately resistant to all four isolates (Table 3). The three pepper cultivars were susceptible to 12889 and OP97, with the exception of Paladin, a partially resistant commercial variety that was resistant to OP97. CM334, a resistant landrace pepper, was resistant

to both 12889 and OP97. None of the pepper or eggplant lines were susceptible to isolates SP98 and SFF3. The final plant health score at the end of the experiment showed significant interactions between lines and isolates (Table 3). There were no significant differences found between repetitions for the first experiment.

In the second experiment, using the most virulent Michigan isolate (12889) from the first study and 10 New York isolates, the

Table 3. Eggplant and pepper lines and cultivars screened for resistance to four Michigan *Phytophthora capsici* isolates obtained from pepper (12889), pickling cucumber (OP97, SFF3), and pumpkin (SP98)^x

Line/ cultivar	Disease response ^y				AUDPC ^z				Plant death (%)			
	12889	OP97	SFF3	SP98	12889	OP97	SP98	SFF3	12889	OP97	SP98	SFF3
Eggplant												
EG-195	R	R	R	R	0 f	0 f	0 f	0 f	0	0	0	0
EG-203	R	R	R	R	0 f	0 f	0 f	0 f	0	0	0	0
Pepper												
Camelot	S	S	R	R	241 a	56 c	6 f	0 f	94	56	0	6
CM334	R	R	R	R	0 f	0 f	0 f	0 f	0	0	0	0
Paladin	S	R	R	R	67 d	6 f	0 f	0 f	63	6	0	0
Red Knight	S	S	R	R	250 a	69 b	0 f	19 e	100	69	19	0

^x AUDPC results were analyzed using ANOVA ($P = 0.05$) and means were separated using Fisher's LSD.

^y The disease response was based on the average plant score at the end of the experiment. If the score was <2 , the line was considered resistant (R); if the score was ≥ 1 but ≤ 2 the line was considered intermediately resistant (IR), if the plant health score was >2 the line was considered susceptible (S).

^z The area under the disease progress curve (AUDPC) was calculated from scores evaluated approximately every 3 days using a 0 to 4 rating scale (0 = healthy, 1 = minor wilting, 2 = moderate wilting, 3 = severe wilting, 4 = plant death).

Table 4. Average area under the disease progress curve (AUDPC) values for *Phytophthora capsici* isolates from Michigan and New York causing crown and root rot symptoms on eggplant and pepper (experiment 2)^z

Isolate	AUDPC		Mean plant health	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
13352	52 d	0 e	0.8	0
13357	95 bc	18 d	1.3	0.8
12889	76 cd	45 c	1.0	1.3
13358	72 cd	52 bc	1.0	1.8
13359	83 cd	62 ab	1.3	1.6
13360	100 abc	74 a	1.6	2.0
13354	107 abc	69 a	1.5	1.8
13353	127 ab	67 ab	1.9	1.9
13351	125 ab	77 a	1.9	2.0
13356	128 ab	77 a	1.8	2.4
13355	135 a	73 a	1.8	2.0

^z This experiment included three eggplant lines (EG195, EG203, and Classic) and one pepper line (Red Knight).

Table 5. The disease response, AUDPC, and plant health of pepper (Red Knight) and eggplant (Classic, EG195, and EG203) lines and cultivars screened for resistance to eleven *Phytophthora capsici* isolates obtained from pepper (12889) and eggplant (13351 to 13360)

Isolate	Mean AUDPC ^w							
	Red Knight		Classic		EG203		EG195	
	Replicate 1 ^x	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
12889	302 abc	152 abc	0 k	27 h	0 k	0 h	0 k	0 k
13351	336 a	163 ab	156 gh	127 cd	8 k	16 h	0 k	0 k
13352	207 defg	0 h	1 k	0 h	0 k	0 h	0 k	0 k
13353	293 abc	178 a	197 defg	86 efg	16 k	2 h	0 k	0 k
13354	327 ab	181 a	103 hij	75 efg	0 k	19 h	0 k	0 k
13355	338 a	182 a	172 fgh	96 def	31 jk	14 h	0 k	0 k
13356	317 ab	183 a	190 efg	102 de	5 k	22 h	0 k	0 k
13357	283 abc	63 g	99 hij	8 h	0 k	0 h	0 k	0 k
13358	255 bcde	139 bc	31 jk	70 fg	0 k	0 h	0 k	0 k
13359	233 cdef	174 a	68 ijk	74 efg	30 jk	0 h	0 k	0 k
13360	267 abcd	184 a	135 ghi	96 def	0 k	1 h	0 k	0 k
Total AUDPC ^y	287 a	145 a	104 b	69 b	8 c	8 c	0 d	0 d
Mean plant health ^z	3.7 S	3.4 S	1.7 IR	2.6 S	0.2 R	0.3 R	0.0 R	0.0 R

^w AUDPC was calculated from scores evaluated every two days using a 0 to 4 rating scale (0 = healthy, 1 = minor wilting, 2 = moderate wilting, 3 = severe wilting, 4 = plant death) numbers represent mean AUDPC from run 1 and run 2, respectively.

^x Experiment replicates were analyzed using ANOVA ($P = 0.05$) and means were separated using Fisher's least significant difference test.

^y Total AUDPC was calculated as the mean AUDPC (calculated from rating scores based on the 0 to 4 rating scale) across all isolates for each line.

^z Mean plant health was calculated as the average plant health value at the end of the study (based on the 0 to 4 rating scale). If the score was <2 , the line was considered resistant (R); if the score was ≥ 1 but ≤ 2 the line was considered intermediately resistant (IR), if the plant health score was >2 the line was considered susceptible (S).

two eggplant breeding lines tested performed better than the susceptible pepper, Red Knight, and the commercial eggplant, Classic. New York isolates 13351, 13353, 13355, and 13356 consistently caused greater wilting on both pepper and eggplant than the Michigan isolate 12889 and New York isolate 13352 (Table 4). Significance of line means did not differ between runs of the experiment, and Red Knight, the susceptible pepper check, was susceptible to all isolates tested, with the exception of 13352 in one run (Table 5). The eggplant cultivar Classic was significantly more susceptible than EG203 and EG194 for 13351, 13353-56, and 13359-60. Eggplant line EG203 was resistant to all isolates except 13356.

Four of the New York isolates of *P. capsici* isolated from eggplants caused significantly more wilting on eggplants than the Michigan isolate 12889. The pepper line, Red Knight, was not significantly more susceptible to any of the New York isolates than the Michigan isolate, 12889.

Discussion

Host resistance is a key component of effective, long-term, sustainable *P. capsici* management and can be achieved by grafting susceptible hosts onto disease-resistant rootstocks. The pepper line CM334 has been extensively tested against isolates worldwide and has demonstrated resistance to isolates of *P. capsici* in addition to other pathogens, but its usefulness as a rootstock is limited (20,27,30,31). The eggplant lines EG195 and EG203 were intermediately to fully resistant to all 14 of the *P. capsici* isolates tested in this study. The eggplant lines appeared to have a similar level of resistance to CM334, with an average final plant health score of 0 or no wilting. Both of the eggplant lines exhibited consistent resistance to *P. capsici* but need to be screened against isolates that represent a greater geographic distribution, due to recognized differentials between isolates (8,21,22,27).

The eggplant lines evaluated were developed as tomato rootstocks, but their compatibility with pepper scions is unknown, in addition to any effects on yield and growth (3). EG195 and EG203 should also be evaluated for tolerance to Verticillium wilt, an important soilborne disease of eggplant, before any further investigation into its potential as a rootstock in Michigan. Prior to this study, there was little information on the resistance of eggplant to *Phytophthora* root rot. Based on these results, eggplant appears to be highly resistant to *P. capsici* root rot and a potential source of resistance.

The differences among Michigan isolates (experiment 1) and between Michigan and New York isolates (experiment 2) are consistent with previous results highlighting the range of virulence in *P. capsici* isolates depending on host and geography (9). Isolate 12889, from pickling cucumber, is highly virulent on peppers and tomatoes (6,22). In this study, isolate 12889 caused almost no disease on the most susceptible eggplant, Classic. The New York isolates from eggplant showed a wide range of virulence, particularly on the eggplant lines. In this study, most eggplant isolates caused more wilting than 12889 on eggplant lines, but not on the pepper line Red Knight, that was susceptible to each isolate tested. Further work with eggplant isolates is needed.

In summary, the eggplant lines were more resistant to *Phytophthora* crown and root rot than the pepper cultivars. EG195 and EG203 were resistant to most of the *P. capsici* isolates screened, and may be potential rootstock sources for field-grown tomatoes, peppers, and eggplants. In addition, *P. capsici* isolates from eggplant caused more wilting on the eggplant lines than on the susceptible pepper (Red Knight), when compared to the Michigan isolate, indicating they might be able to cause more wilting. Future research to test the virulence of eggplant isolates on other solanaceous species will provide more information for understanding host preference within *P. capsici*. Further research is also warranted to test EG195 for resistance to additional *P. capsici* isolates and to evaluate it for potential rootstock compatibility to pepper.

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