Screening Tomato Seedlings for Resistance to *Verticillium dahliae* Races 1 and 2

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ABSTRACT

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Tomato (*Lycopersicon esculentum*) cultivars and breeding lines were screened in the greenhouse for resistance to local isolates of *Verticillium dahliae* races 1 and 2. Heinz 1350, C28, Morden MEL2668170G, and Morden LAC3684 were most resistant to race 2, although the level of resistance was less than that conferred by the *Ve* gene against race 1. Italian Winter was highly susceptible to both races. Correlation of parental and F_1-array means of 10 selected lines crossed in a half diallel was highly significant for both races. The F_1 most resistant to race 2 was Morden MEL2668170G × Morden LAC3684. F_1 hybrids heterozygous for the *Ve* gene were less resistant to race 1 than homozygous F_1 hybrids, suggesting incomplete dominance of the *Ve* gene.

Verticillium wilt (VW), incited either by *Verticillium albo-atrum* Reinke & Berth. or *V. dahliae* Kleb., is a severe disease of tomato (*Lycopersicon esculentum* Mill.) in California (16), Florida (7), Ohio (1), and North Carolina (2). The *Ve* gene for resistance to race 1 (11) is now widely used in these areas. Tolerance has also been reported (7,8,15).

As early as 1957, *Verticillium* isolates from tomatoes and potatoes were reported pathogenic on Loran Blood, one of the original *Ve* tomato cultivars (10). By 1962, race 2 was causing VW on tomatoes resistant to race 1 in Ohio (1). Race 2 is now a serious problem in western North Carolina, where in 1980 race 2 was apparent in two-thirds of the unfumigated fields (P. B. Shoemaker, *personal communication*), and in California, where the continued use of *Ve* cultivars has resulted in 100% infection in some fields (5). Four other countries have also reported race 2 (4,9,14). No tomato cultivars resistant to race 2 are available, although Heinz 1350 has been reported to be "field resistant" (6). No high levels of resistance to California isolates of race 2 have been reported in tests of many wild and primitive *Lycopersicon* accessions (5,12).

The purpose of this research was to screen tomato germ plasm for resistance or tolerance to North Carolina isolates of race 2 of *V. dahliae*. Some entries were also tested with race 1 to verify the action of the *Ve* gene.

MATERIALS AND METHODS

We tested 141 tomato lines of diverse origin, including those reported as tolerant or moderately resistant to VW (presumably race 1) and for which seed was available from the Plant Gene Resources of Canada (Ottawa Research Station, Ottawa, Ontario), the USDA Regional Plant Introduction Station (Ames, IA), and from seed companies and tomato breeders. Seed of some entries was increased by collecting fruit of single plants grown in the greenhouse. Ten selected lines—Bonny Best (BB),

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Italian Winter (IW), June Pink (JP), Morden LAC3684 (LAC), Morden MEL2668170G (MEL), Walter (WA), Flora-Dade (FD), Ottawa 112 (OTT), VF145B-7879 (VF), and 139-1 (AWA)—were crossed in a half diallel and the parents and F_1 progenies were screened for resistance to race 1 and race 2. Seed of reciprocal F_1 crosses was pooled.

Race 1 and race 2 isolates collected by C. G. Bender from tomato fields in western North Carolina were used in addition to race 1 isolate CF from Tennessee. Race 2 isolates 91A and 105A and race 1 isolate 20B were used most often. For each isolate, an inoculum slurry (11) was prepared by blending the contents of 2-wk-old cultures, grown on potato-dextrose agar in 9-cm-diameter petri plates, with an equal volume of distilled water.

Seedlings were screened by a standard root-dip technique (11) in a greenhouse at the Mountain Horticultural Crops Research Station, Fletcher, NC, from 1977 to 1979. Seeds were sown in metal flats filled with a pasteurized medium consisting of vermiculite:peat:sand:loam (10:12:10:3) plus 7.5 kg of dolomitic lime, 1.2 kg of 20% superphosphate, 1.0 kg of potassium nitrate, 295 g of magnesium sulfate, 37 g of iron chelate, 10 g of borax, and 111 g of FRIT 503 fritted trace elements per cubic meter. Seedlings 10–14 days old, most showing the first true leaf, were inoculated. Excess soil mix was brushed from the roots, which were trimmed to a 3-cm length before being immersed in inoculum for 10 min. Control plants were dipped into an agar slurry or into tap water.

Seedlings were then transplanted 8 cm apart in plastic or metal flats, with four-plant subplots of each of six entries randomized within each flat. Main plots, groups of flats inoculated with the same isolate, were randomized in three to six blocks per test. Our preliminary results and other reports (5,13) indicate that race 2 ratings based on reduction in plant height, degree of vascular discoloration, or presence of the pathogen within the stem are not as reliable in measuring disease susceptibility as are ratings based on foliar symptoms. Plants were thus rated periodically for about 7 wk by counting cotyledons and leaves showing symptoms of wilting and chlorosis. For race 1 isolate 20B, which often killed plants, a maximum score of 9 (dead) was used. Preplanned linear contrasts were used to compare parental lines and F_1 progenies in the diallel test.

RESULTS

An initial comparison of 12 lines inoculated with each of seven race 2 isolates showed no significant line × isolate interactions. Because the isolates varied in virulence but not pathogenicity, only the most virulent were used in subsequent tests. Race 2 isolates consistently produced symptoms of wilting and chlorosis on race 1 resistant (*Ve*) cultivars. On lines susceptible to race 1, race 2 isolates produced symptoms almost as severe as those of race 1 isolate CF but much milder than those of 20B. An inoculated plant of an otherwise susceptible line occasionally remained disease-free, but symptoms developed after reinoculation. VW symptoms were not seen on control plants.

A complete list of the scores and sources of the tested lines is available from the junior author. The reactions of 41 lines (Table 1) and of the 10 selected parental lines (diagonal of Table 2) to inoculation with race 2 isolates 91A and 105A are given here. The correlation between race 2 scores for the 10 lines in common was significant ($r = 0.75$, $P = 0.01$), as was the correlation ($r = 0.50$, $P = 0.01$) of the parental scores and their F_1-array means in the diallel (Table 2). A linear contrast comparing means for BB, JP, and IW with the means of their outcrossed progenies was significant ($P = 0.05$). No F_1 was as susceptible as IW. A contrast of the means for MEL and LAC with the means of their outcrossed F_1 progenies was also significant. In addition, the scores of outcrossed F_1 progeny of MEL and LAC were significantly lower than those of progeny of BB, JP, and IW.

Race 1 isolate 20B produced severe symptoms on lines not carrying the *Ve* gene; death often occurred within 3 wk of inoculation. Experimental race 1 reac-

Table 1. Reported and experimental reactions of tomato lines inoculated as seedlings with North Carolina isolates of *Verticillium dahliae* races 1 and 2

Line	Reported reaction[a]	Experimental reaction	
		Race 2[b]	Race 1[a]
Italian Winter		8.9	S
San Marzana	R	8.6	S
Marmande V	R	8.5	G
June Pink		8.3	S
Bonny Best		8.3	S
Merveille des Marches	T	8.3	
Sub-Arctic Delight		8.2	
Peru Wild (Utah 665)	R	8.1	S
Manx Marvel	T	8.0	
PI 167103	R	7.9	S
Lipso F_1	R	7.9	R
Simi	T	7.8	
Pearson	T	7.8	
Saladmaster	R	7.8	R
Rutgers	T	7.7	
Morden IH1682	MS	7.6	
Early Santa Clara Canner	T	7.4	S
Loran Blood	R	7.3	S
Moscow VR	R	7.2	R
Campbell Soup 119	T	7.1	
Sub-Arctic Maxi		6.6	
Flora-Dade	R	6.6	R
Walter	T	6.4	MR
Morden MEL2668170G	MR	6.4	S
Ottawa 112	MR	6.4	G
Stakeless		6.3	S
Marvel		6.2	
PI 272790	T	5.9	
Morden 67T95683		5.9	
Croja	R	5.9	R
Morden 67T941170G		5.6	
139-1	R	5.6	R
Morden LAC3684	MR	5.5	S
Morden MEL2668170G (×)[c]		5.5	S
VF145B-7879	R	5.4	R
Morden LAC3682	MR	5.2	
Morden LAC3684 × Morden MEL2668170G F_1		4.9	S
Caro-Rich		4.6	R
Morden LAC3684 (×)[c]		4.5	S
C28		4.3	
Heinz 1350	T2,R	4.0	
LSD[d]		0.7	

[a] R = resistant, MR = moderately resistant, T = tolerant, MS = moderately susceptible, S = susceptible, G = segregating for resistance, T2 = tolerant to race 2. Reported reactions, except T2, presumably refer to race 1. Experimental race 1 reactions are composites of several tests.

[b] Mean number of leaves and cotyledons showing wilt symptoms 7 wk after inoculation with a mixture of isolates 91A and 105A; six replicates of four plants each.

[c] Selfed two times.

[d] Waller-Duncan K-ratio (K = 100) least significant difference for comparing any two means.

tions, listed along with published reactions, were the result of several screenings (Table 1). Resistant lines were those with mean ratings of 0–2; moderately resistant, 2–5; and susceptible, greater than 5. Segregating lines had substantial numbers of plants with resistant and susceptible ratings. Within susceptible lines, single plants sometimes escaped infection, although less often than with race 2. Some plants of resistant lines showed very mild symptoms. The correlation of parental and F_1-array means for resistance to race 1 (Table 3) was significant ($r = 0.90$, $P = 0.01$). A linear contrast comparing means of *VeVe* hybrids with those of *Veve* hybrids was also highly significant ($P = 0.01$). OTT was not included in contrasts of *VeVe*-type parents, but F_1 progeny of OTT were included in all appropriate contrasts because *Ve* gene segregation for them appeared normal.

DISCUSSION

Plants in tests conducted between late fall and early spring had more severe symptoms with fewer escapes than those in summer tests. The escapes may have been caused by interfering soil microflora (16) or may have been related to plant size at inoculation. Although small plants have a longer time to develop symptoms before becoming overcrowded in the flat, those less than a week old did not develop symptoms uniformly. Seedlings of OTT, WA, BB × OTT, and FD × JP were late to emerge and so were very small at the time of inoculation. Their race 2 scores were low compared with previous tests and with the other F_1 progeny in the diallel (Table 2).

Race 2 ratings in Table 1 are similar to those in our earlier tests. However, Heinz 1350, C28, and Caro-Rich, reported as significantly more resistant than MEL in Table 1, were less resistant in earlier tests.

Table 2. Mean Verticillium wilt scores and F_1 array means for seedlings of 10 tomato parents and 44 hybrids inoculated with race 2 isolate 91A of *V. dahliae*

	Parent									
Parent[a]	MEL	LAC	OTT	WA	VF	FD	AWA	JP	BB	IW
MEL	2.8[b]	1.6	3.3	4.1	4.1	3.8	4.3	3.9	4.5	5.3
LAC		3.9	3.4	3.5	3.8		3.9	3.3	3.9	4.9
OTT			3.0	3.6	3.7	3.4	3.4	3.9	3.8	4.9
WA				3.4	4.0	3.2	3.9	4.5	4.5	5.2
VF					4.2	3.9	4.0	4.2	4.1	5.3
FD						3.8	3.6	2.8	4.5	5.0
AWA							3.8	4.5	4.5	5.1
JP								5.0	4.3	5.2
BB									4.9	5.5
IW										6.0
F_1 mean	3.9	3.5	3.7	4.1	4.1	3.8	4.1	4.1	4.4	5.2

[a] MEL = Morden MEL2668170G, LAC = Morden LAC3684, OTT = Ottawa 112, WA = Walter, VF = VF145B-7879, FD = Flora-Dade, AWA = 139-1, JP = June Pink, BB = Bonny Best, and IW = Italian Winter.

[b] Mean number of leaves and cotyledons with symptoms 5 wk after inoculation, for six replicates of four plants each. Error mean square = 0.4.

Table 3. Mean Verticillium wilt scores and F_1 array means for seedlings of 10 tomato parents and 43 hybrids inoculated with race 1 isolate 20B of *V. dahliae*

	Parent									
Parent[a]	MEL	LAC	OTT	WA	VF	FD	AWA	JP	BB	IW
MEL	7.1[b]	6.5	2.2	6.6	1.8	0.6	2.2	6.3	7.1	8.0
LAC		6.3	3.2	5.6	4.6		0.8	5.7	7.2	7.1
OTT			4.5	1.6	0.4	1.3	0.4	2.1	2.4	1.0
WA				3.9	0.8	0.5	2.2	4.1	4.5	5.7
VF					1.0	0.8	0.5	1.1	1.3	4.4
FD						0.7	1.0		2.4	1.2
AWA							0.8	3.2	1.2	2.7
JP								7.7	6.4	7.1
BB									8.5	6.7
IW										8.6
F_1 mean	4.6	5.1	1.6	3.5	1.7	1.1	1.6	4.5	4.4	4.9
VeVe F_1 progeny			0.7		0.6	1.0	0.6			
Veve F_1 progeny	1.7	2.9	2.1	1.3	2.3	1.2	2.1	2.1	1.9	2.3
veve F_1 progeny	6.9	6.4		5.3				5.9	6.4	6.9

[a] MEL = Morden MEL2668170G, LAC = Morden LAC3684, OTT = Ottawa 112, WA = Walter, VF = VF145B-7879, FD = Flora-Dade, AWA = 139-1, JP = June Pink, BB = Bonny Best, IW = Italian Winter.

[b] Rated (0 = healthy to 9 = dead) 5 wk after inoculation; three replicates of four plants each. Error mean square = 1.7.

WA , Heinz 1350, Campbell Soup 119, and PI 272790 exhibited some resistance in all tests, confirming earlier reports (3,6,7). In contrast, Simi, Pearson, Manx Marvel, and Rutgers were more susceptible than previously reported (8,15), although they may possess specific tolerance to race 1.

Multiple tests on the 10 selected lines showed four overlapping classes of reaction to race 2: highly susceptible (IW), susceptible (BB, JP), slightly resistant (WA, FD, VF, AWA), and moderately resistant (LAC, MEL, OTT). Symptoms on MEL and LAC were milder than their scores indicated because individual leaves often showed milder symptoms than leaves of susceptible plants rated similarly. Few, if any, F_1 crosses were as resistant as MEL. LAC × MEL was as resistant as MEL in the diallel test, but it was rated slightly more resistant than MEL and equal to LAC in a later test (Table 1). Both lines are from the cross (Earlinorth × Bush Beefsteak) × Earlinorth and may not be completely homozygous, causing some variation in their scores. Leaves of OTT never showed chlorosis; any stress caused leaf necrosis, apparently the result of its mutant color genes (probably *hp, gf, og*^{c}). Outcrossed F_1 progeny of OTT showed chlorosis, yet scored low; some true resistance to race 2 may thus have been involved.

Earlier field observations of F_1 hybrids suggested that lines homozygous for the *Ve* gene were more resistant to race 1 than those heterozygous. The greater susceptibility of *VeVe* heterozygotes in the greenhouse (Table 3) appeared in terms of plants rated 3–5, and only rarely as highly susceptible plants. If pollen contamination had occurred, the highly susceptible *veve* genotype would have resulted. These results indicate that the *Ve* gene was not completely dominant under the severe disease pressure in this test.

The *Ve* gene does not appear to affect resistance to race 2. BB, JP, and IW were susceptible to both races, whereas MEL and LAC were susceptible only to race 1. Among the *Ve* cultivars, Lipso F_1 and Saladmaster were susceptible to race 2, whereas Heinz 1350 and perhaps OTT were somewhat resistant to race 2. The moderate resistance of WA also appeared in its F_1 progenies; its F_1-array means were among the lowest for both *Veve* and *veve* classes (Table 3). The resistance carried by WA did not appear to be race specific, although it was more obvious against the virulent race 1 isolate.

Seedling screening is most effective when high levels of both host resistance and pathogen virulence are available, such as with VW race 1. The moderate resistance and virulence available for race 2 are disadvantages that can be partly overcome by using multiple tests to reduce environmental effects. The resistance of MEL and LAC to race 2 is

the best now available, and the significant correlation coefficients and contrasts from the diallel indicate that genetic effects are involved. Seedling screening should allow the recovery of lines with resistance to both races. These lines can then be field tested to see if this resistance to race 2 is adequate for commercial use.

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LITERATURE CITED

1. Alexander, L. J. 1962. Susceptibility of certain *Verticillium*-resistant tomato varieties to an Ohio isolate of the pathogen. Phytopathology 52:998-1000.
2. Bender, C. G., and Shoemaker, P. B. 1977. Prevalence and severity of Verticillium wilt of tomato and virulence of *Verticillium dahliae* Kleb. isolates in western North Carolina. (Abstr.) Proc. Am. Phytopathol. Soc. 4:152.
3. Clark, R. L., Jarvis, J. L., Braverman, S. W., Dietz, S. M., Sowell, G., Jr., and Winters, H. F. 1975. A summary of reports on the resistance of plant introductions to diseases, nematodes, insects, mites and chemicals: *Lycopersicon* spp. U.S. Dep. Agric. Reg. Plant Introd. Stn., Ames, IA. 70 pp.
4. de Badilla, H. S. 1974. Estudio preliminar de resistencia de lineas y/o variedades de tomate a *Verticillium albo-atrum* Reinke et Berth. Agric. Tec. (Santiago) 34:39-40.
5. Grogan, R. G., Ioannou, N., Schneider, R. W., Sall, M. A., and Kimble, K. A. 1979. Verticillium wilt on resistant tomato cultivars in California: Virulence of isolates from plants and soil and relationship of inoculum density to disease incidence. Phytopathology 69:1176-1180.
6. Hubbeling, N., Alexander, L. J., and Cirulli, M. 1971. Resistance to Fusarium and Verticillium wilts in tomato. Meded. Rijksfac. Landbouwwet Gent 36:1006-1016.
7. Jones, J. P., and Crill, P. 1975. Reaction of resistant, tolerant, and susceptible tomato varieties to Verticillium wilt. Plant Dis. Rep. 59:3-6.
8. Lesley, J. W., Middleton, J. T., and McCarty, C. D. 1952. Simi, a processing tomato resistant to Verticillium and Fusarium wilts. Hilgardia 21:289-299.
9. Pegg, G. F. 1974. Verticillium diseases. Rev. Plant Pathol. 53:157-182.
10. Robinson, D. B., Larson, R. H., and Walker, J. C. 1957. Verticillium wilt of potato in relation to symptoms, epidemiology, and variability of the pathogen. Univ. Wis. Agric. Exp. Stn. Res. Bull. 202. 49 pp.
11. Schaible, L., Cannon, O. S., and Waddoups, V. 1951. Inheritance of resistance to Verticillium wilt in a tomato cross. Phytopathology 41:986-990.
12. Stevens, M. A. 1979. Breeding tomatoes for processing. Pages 201-213 in: Proc. Int. Symp. Trop. Tomato, 1st. Shanhua, Taiwan, Republic of China. 23–27 October 1978. Asian Veg. Res. Dev. Cent. Pub. 78-59.
13. Thanassoulopoulos, C. C., and Kitsos, G. T. 1974. Stem length of tomato for evaluating the severity of attack by Verticillium wilt. Phytopathol. Z. 81:258-261.
14. Tjamos, E. C. 1981. Virulence of *Verticillium dahliae* and *V. albo-atrum* isolates in tomato seedlings in relation to their host of origin and the applied cropping system. Phytopathology 71:98-100.
15. Walter, J. M. 1967. Hereditary resistance to disease in tomato. Annu. Rev. Phytopathol. 5:131-162.
16. Wilhelm, S. 1975. Sources and nature of Verticillium wilt resistance in some major crops. Pages 166-171 in: G. W. Bruehl, ed. Biology and Control of Soil-borne Plant Pathogens. Am. Phytopathol. Soc., St. Paul, MN.