Differential Phytotoxicity of Peptides from Culture Fluids of *Verticillium dahliae* Races 1 and 2 and Their Relationship to Pathogenicity of the Fungi on Tomato

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ABSTRACT

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Confirming other work, we found that race 2 isolates of *Verticillium dahliae* caused severe symptoms on tomato plants carrying the *Ve* gene for Verticillium resistance, whereas race 1 isolates caused little or no damage. However, both fungi colonized *Ve* tomato stems and attained similar populations. Phytotoxic peptides were isolated from the culture fluids of *V. dahliae* races 1 and 2 and were found to differ in amino acid composition and toxicity to tomato leaves, root tips, and suspension cells. The race 1 peptide produced more severe symptoms in plants lacking the *Ve* gene than those that contained the *Ve* gene. On the other hand, the peptide from race 2 isolates produced indistinguishable, severe symptoms on both tomato genotypes. The results therefore suggest that the *Ve* gene may function by conferring tolerance to the phytotoxic effects of the race 1 peptide. The pathogenicity of race 2 isolates on *Ve* tomato plants may be due to their production of an altered peptide toxin, which causes severe effects on plants carrying the *Ve* gene as well as those lacking this gene.

Additional key words: disease resistance genes, toxins, vascular wilt disease.

Verticillium wilt disease, caused by *Verticillium dahliae* Kleb. or *V. albo-atrum* Reinke & Berth., is a major limiting factor in tomato (*Lycopersicon esculentum* L.) production in several areas of the United States (1,4,8,12,23). Yield losses in cultivars susceptible to Verticillium wilt sometimes are as high as 30–70% (2,6,12), but the disease may be controlled by planting disease-resistant cultivars. Resistance to Verticillium wilt in tomato is conferred by a single dominant gene (*Ve*) (18), which is carried by most commercial tomato cultivars worldwide. The appearance of a Verticillium isolate pathogenic on tomato cultivars possessing the *Ve* gene was first reported in Wisconsin in 1957 (17). This new biotype, designated race 2, has become a serious problem in California (8,9) and North Carolina (4).

Race 1 of *V. dahliae* isolated from potato produces a phytotoxic peptide in culture, which was associated with the production of wilt symptoms in numerous hosts (5,16), including tomato plants lacking the *Ve* gene. The peptide was not phytotoxic to tomato cultivars containing the *Ve* gene (16), suggesting that it might be involved in host specificity. The aim of this work was to investigate if races 1 and 2 of *V. dahliae* isolated from tomato produce toxic peptides with differential activity and, if so, to investigate if the differential pathogenicity of the two races on *Ve* tomato plants might be related to differences in the composition of their respective toxins. To further investigate this possibility, we purified the peptide toxins from races 1 and 2 of *V. dahliae* and determined their amino acid composition and biological activity on *ve* and *Ve* tomato cells.

MATERIALS AND METHODS

Plant material. The near-isogenic tomato lines, cultivar Roma F (*ve*, susceptible to *V. dahliae*) and cultivar Roma VF (*Ve*, resistant to *V. dahliae* race 1), were supplied by Dr. Jon Watterson, Peto Seed Co., Inc., Woodland, CA. Other cultivars were obtained commercially.

Fungal material. A race 1 potato strain of *V. dahliae* (Dvir I) was isolated from potato cultivar Desiree. The race 1 tomato isolate ATR-13 was supplied by Watterson, Peto Seed Co., Inc., Woodland, CA. A race 2 tomato isolate (Ruhama 2) was supplied by Mark Martin, Prosser, WA, and isolates 50A, 20B, and 91A were supplied by Dr. P. B. Shoemaker, North Carolina State University, Raleigh.

Inoculation procedures. Tomato plants were grown on sterile vermiculite. When four true leaves were present, the roots were dipped into a *V. dahliae* spore suspension (5×10^4 spores per milliliter) for 30 min and planted in a soil/peat/vermiculite mixture (1:1:1). Microsclerotia were produced on PDA medium in petri dishes, thoroughly blended with soil mixture, and air-dried at room temperature for 2 wk. Seeds were then sown in the mixture and plants were grown under greenhouse conditions at about 28 C for 45 days.

Fungal populations in plants. Plants were excised and 5-cm stem segments were prepared. The surface was sterilized by dipping in 1.5% sodium hypochlorite for 10 min, then washed thoroughly with sterile distilled water. Three 1-cm segments from each of five replicate plants were blended for 2 min with 10 ml of sterile distilled water in a Sorvall Omnimixer. Serial dilutions were made from the mixture and spread uniformly onto plates containing 0.2% sorbose and 100 ppm of streptomycin sulfate. The plates were incubated in the dark at 25 C for 10–14 days and *V. dahliae* colonies counted. Populations were calculated as colony-forming units obtained per 1-cm stem segment.

Toxin production. Cultures were grown and resulting filtrates partially purified by acetone precipitation, agarose gel filtration, and dialysis as previously described (5,15). For production of the toxin, the fungi were grown in 100 ml of medium containing 2 g of glucose, 0.2 g of asparagine, 0.15 g of $KH_2PO_4 \cdot 7H_2O$, 1 mg of $FeSO_4 \cdot 5H_2O$, 0.6 mg of $CaSO_4$, 1 mg of thiamine HCl, and 0.5 mg of pyridoxine; pH was adjusted to 6.7 with KOH, and the medium was autoclaved. The cultures were incubated for 21 days at 26 C in the dark. High-performance liquid chromatography (HPLC) was performed on a Spectraphysics 8100 liquid chromatograph with a 250×4.6-mm column of Nucleosil 5 μm (Macherey-Nagel, Duren,

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West Germany). The column was eluted at room temperature at a flow rate of 0.5 ml min^{-1} using a linear gradient of 0–10% *n*-propanol in 0.1% trifluoroacetic acid. The column effluent was monitored by absorbance at 210 nm. Fractions were assayed for phytotoxicity by the detached leaf bioassay. All toxin preparations used in this work were purified as shown in Figure 1. Amino acid analyses were performed on highly purified fractions.

Amino acid analyses. Toxin preparations were hydrolyzed in 6 N HCl at 110 C for 22 hr under vacuum and analyzed with a Biotronik (Puchheim/Obb, West Germany) automatic amino acid analyzer following the procedures of Hare (10).

Toxin bioassay. *Detached leaf assay.* Plants were screened for toxin sensitivity by a detached leaf assay using potato (15) or tomato leaves. The first true leaves of 3–6-wk-old plants were excised under water and placed in vials containing 20 ml of water. Test solutions (0.1 ml) were injected into the intercellular spaces of the leaves using a 22-gauge needle. The cuttings were placed under continuous illumination (about 7,000 lx) for 18–36 hr, after which the extent of chlorosis and necrosis was recorded by visual observation.

Root tip assay. Root tips were obtained by germinating surface-sterilized seeds (70% ethanol for 1 min followed by 1.5% sodium hypochlorite for 10 min) in sterile distilled water. Ten root tips (0.5–1 cm long) were excised and placed into a depression slide with 0.2 ml of MS salt medium, pH 5.8 (7). This medium included, per liter: 1,650 mg of NH_4NO_3, 1,900 mg of KNO_3, 440 mg of $CaCl_2$, 370 mg of $MgSO_4$, 170 mg of KH_2PO_4, 0.8 mg of KI, 6.2 H_3BO_3, 22.3 mg of $MnSO_4{\cdot}4H_2O$, 8.6 mg of $ZnSO_4{\cdot}7H_2O$, 0.25 mg of $Na_2MoO_4{\cdot}2H_2O$, 0.025 mg of $CuSO_4$, 0.025 mg of $CoCl_2$, 37.3 mg of $Na_2{\cdot}EDTA$, and 27.8 mg of $FeSO_4{\cdot}7H_2O$. Toxin was added at various concentrations and the slides incubated for 6 hr in a moist chamber at room temperature. After the incubation period, a 0.01% solution of sodium fluorescein was added and mixed gently. After 1 hr the staining solution was removed with a hypodermic syringe and the stained tissue washed three times with MS salt medium for 10 min each. The roots were viewed with a Zeiss Photomicroscope III under incident light from an HB050 mercury-vapor lamp. Excitation filter KP490 was used in combination with barrier filter LP528. At least 10 root tips from three replicate treatments were observed.

Tomato cell suspension assay. Seeds of *L. esculentum* cultivars Roma F and Roma VF were grown under sterile conditions in test tubes (25 mm × 15 cm) on MS media at 21 C with a light intensity of 4,000 lx and a 16-hr day length. Callus was then initiated from leaves of the two cultivars by placing 0.5- × 0.5-cm pieces onto B-5 medium as described by Gamborg and Wetter (7) and incubating at 27 C in the dark. Undifferentiated callus was transferred three times on the B-5 agar medium containing 10 $\mu g\ ml^{-1}$ 2,4-D and then resuspended in the same medium (25 ml per 125-ml Erlenmeyer flask) without agar and shaken on a rotary shaker at 150 rpm at 22 C. After five or more sequential transfers, cell suspensions were used for toxin bioassays. *L. esculentum* suspension cells (0.1 ml containing about 10^4 cells) were added to each well of a microtiter plate (Dynatech Labs Inc.). A sterile toxin preparation dissolved in B-5 medium was added to each well to give a final volume of 0.2 ml. The mixture was incubated for 4 hr at room temperature with gentle agitation every 15 min. After the incubation period, 0.1 ml of the medium was removed with a syringe and 26-gauge needle and 0.1 ml of sodium fluorescein in B-5 salt medium was added to give a final fluorescein concentration of 0.01%. After incubation for 1 hr at room temperature, the medium containing the toxin and stain was removed with a syringe and needle as before and the cells were washed twice with fresh B-5 medium lacking sodium fluorescein. An aliquot of the cell suspension was taken for microscopic analysis as in the root tip assay. The percentage of viable cells was determined based on at least 100 cells observed. Cells that failed to fluoresce were assumed to be dead.

RESULTS

Differential pathogenicity of races 1 and 2 was observed on susceptible and race 1-resistant (*Ve*) tomato cultivars inoculated by the root dip method (Table 1) or by soil infestation (Table 2). Race 1 was not pathogenic on *Ve* cultivars, but the race 2 isolates produced symptoms on all of them. However, race 1 was isolated from stems of tomato cultivars carrying the *Ve* gene and yielded nearly as many colony-forming units as the race 2 isolates (Tables 1 and 2).

Peptides purified from races 1 and 2 by HPLC eluted with very similar retention times (Fig. 1), but differed in amino acid composition. The peptide from race 2 contained extra residues of aspartic acid and tyrosine, but one less glutamic acid residue than the peptide from race 1; the race 2 peptide had no glycine (Table 3). Both purified peptides produced similar symptoms on potato leaflets, including interveinal chlorosis followed by necrosis. No differences were noted in the concentration potency of the two preparations on potato (data not shown). In tomato leaves, however, the peptide toxin from race 1 was active on susceptible cultivars at 5 $\mu g\ ml^{-1}$, but gave no visible symptoms on cultivars carrying the *Ve* gene (Table 4). The corresponding race 2 peptides from three isolates caused similar symptoms on all tested cultivars at 3 and 5 $\mu g\ ml^{-1}$.

The purified peptides also caused necrosis of tomato root cells as determined by the absence of sodium fluorescein uptake, but this

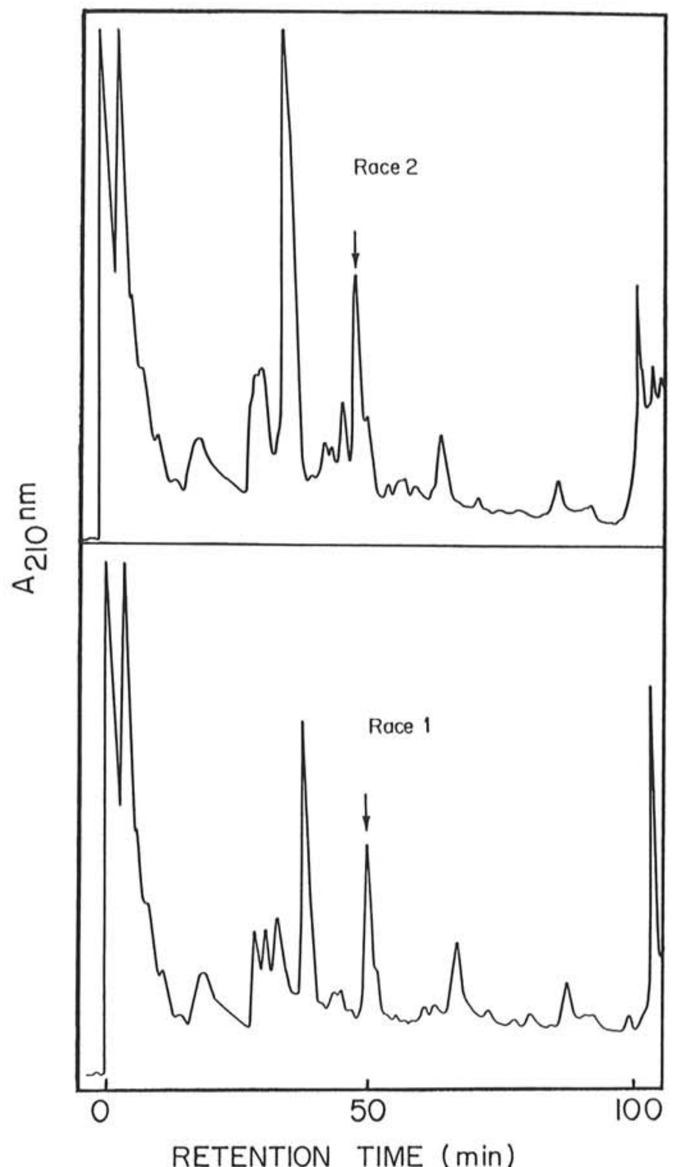

Fig. 1. HPLC chromatogram of partially purified peptide toxin from *Verticillium dahliae* race 1 (lower) and race 2 (upper). The peaks denoted by arrows produced Verticillium wilt symptoms when injected into leaflets of tomato cultivars lacking the *Ve* gene (race 1) and in cultivars lacking or containing the *Ve* gene (race 2).

assay was only useable within a relatively narrow concentration range. At 10 $\mu g\ ml^{-1}$ and higher, both race 1 and 2 peptides exhibited toxic effects on all tested tomato cultivars (Table 5), whereas 0.5 $\mu g\ ml^{-1}$ or less did not cause significant phytotoxicity on any cultivar. At 5 $\mu g\ ml^{-1}$, however, the race 1 peptide caused substantial cell death on susceptible cultivars but not on two different *Ve* cultivars.

A more quantitative bioassay was devised in which toxin preparations were incubated with suspension cells of the near-isogenic cultivars Roma F and Roma VF. In all cases, 10 $\mu g\ ml^{-1}$ of toxin caused extensive cell death, but the *Ve* line (Roma VF) showed less damage than Roma F with the race 1 peptide. At 1–5 $\mu g\ ml^{-1}$, the race 1 peptide caused almost no damage to Roma VF cells, but substantially reduced the viability of Roma F cells. The race 2 peptide at 1–5 $\mu g\ ml^{-1}$ reduced the viability of cells of both cultivars by 90% or more (Table 6, Fig. 1). The suspension cell assay therefore appears to be a sensitive and quantitative measure of differential toxicity caused by the purified *V. dahliae* peptides.

DISCUSSION

The results presented here confirm those of Bender and Shoemaker (4) indicating that tomato races 1 and 2 of *V. dahliae* show differential pathogenicity on resistant (*Ve*) tomato cultivars. No significant differences in isolable colony-forming units were found in resistant and susceptible plants inoculated by two different techniques (Tables 1 and 2), but symptoms were generally not observed on *Ve* tomato plants inoculated with race 1. In one case, slight disease symptoms were observed in cultivar VF134-1 (Table 1). Although considerable error can occur in attempts to quantify the populations of vascular wilt fungi, it is clear that race 1 does extensively colonize *Ve* plants despite the general absence of disease symptoms. Thus, instead of large differences in fungal populations in *ve* and *Ve* tomato plants, we noted that tomato cultivars lacking the *Ve* gene were more sensitive in three different assays to the race 1 peptide than were *Ve* gene cultivars. On the other hand, race 2 isolates produced a different peptide toxin (Table 3), which damaged tomato plants irrespective of genotype (Tables 5 and 6). These data suggest that the *Ve* gene has little or no effect on *V. dahliae* multiplication in the plant but instead limits damage. Although our results should be treated with some caution because yield data were not obtained and the differences in response to the toxins was only about 10 ×, the data confirm and extend several previous reports that differential activity of a *V. dahliae* toxin is associated with the pathogenicity of the fungus on various host cultivars (5,13,16).

The differential activity of the toxic peptide from tomato race 1

TABLE 1. Colony-forming units isolated and disease symptoms following inoculation of four tomato cultivars with races 1 and 2 of *Verticillium dahliae* by the root dip method[a]

Cultivar	Colony-forming units and symptoms: Race 1 isolates ATR-13	20A	Race 2 isolates Ruhama 2	50A
Roma	6×10^{4}[b] (3)[c]	4.7×10^{2} (2)	$3 \times 2 \times 10^{4}$ (3)	3.1×10^{3} (2)
Hosen Eilon	5.7×10^{3} (3)	2.6×10^{3} (3)	8.1×10^{3} (3)	6.3×10^{2} (3)
Roma VF	1.8×10^{2} (0)	2.4×10^{3} (0)	7.5×10^{3} (2.5)	4.9×10^{3} (2)
VF-134	2.0×10^{2} (0.5)	3.1×10^{3} (0)	4.1×10^{2} (2)	9.4×10^{3} (2)

[a]Tomato seedlings at the four true-leaf stage were dipped in a *Verticillium dahliae* spore suspension (5×10^{4} spores per milliliter) and planted in soil/peat/vermiculite.
[b]Colony-forming units isolated from 1-cm segments of tomato stems 29 days after inoculation; segments excised from the fourth internode in all cases.
[c]Observed symptoms in parentheses: 0 = no symptoms; 1 = leaves chlorotic; 2 = leaves chlorotic and necrotic; 3 = leaves wilted, severely chlorotic, and necrotic.

TABLE 2. Colony-forming units isolated and disease symptoms following inoculation of four tomato cultivars with race 1 or 2 of *Verticillium dahliae* by planting seeds in soil infested with microsclerotia[a]

Cultivars	Colony-forming units and symptoms: Race 1 isolate Dvir 1	Race 2 isolate Ruhama 2
Roma F	2×10^{2}[b] (2.5)[c]	0.1×10^{2} (2)
Hosen Eilon	1.7×10^{2} (3)	0.9×10^{2} (3)
Roma VF	0.4×10^{2} (0)	1.4×10^{2} (2)
VF-134	0.8×10^{2} (0)	3.4×10^{2} (3)

[a]Tomato seeds planted in a soil/peat mixture infested with *V. dahliae* microsclerotia (75 per gram of soil) in the greenhouse. Data taken 20 days after planting.
[b]Colony-forming units isolated from 1-cm segment of tomato stems 29 days after inoculation; segments excised from the fourth internode in all cases.
[c]Observed symptoms; ratings as in Table 1.

TABLE 3. Amino acid composition of peptide toxins isolated from culture fluids of *Verticillium dahliae* races 1 or 2 and highly purified by HPLC

Amino acid	Race 1 (ATR-13) pmol[a]	Race 1 (ATR-13) Residues[b] (no.)	Race 2 (Ruhama 2) pmol[a]	Race 2 (Ruhama 2) Residues[b] (no.)
Aspartic acid	48	1	236	2
Threonine	45	1	74	1
Serine	54	1	71	1
Glutamic acid	93	2	91	1
Proline	ND[c]	ND	ND	ND
Glycine	48	1	50	0
Alanine	44	1	138	1
Cysteine	ND	ND	ND	ND
Valine	40	*1*	106	*1*
Methionine	0	0	0	0
Isoleucine	22	0	63	0
Leucine	31	1	94	1
Tyrosine	13	0	87	1
Phenylalanine	76	2	173	2
Lysine	0	0	11	0
Histidine	0	0	29	0
Arginine	ND	ND	ND	ND

[a]Picomoles detected.
[b]For estimation of residues, valine arbitrarily chosen as 1.
[c]ND = not determined by this procedure.

TABLE 4. Biological activity of the purified peptide toxins isolated from races 1 and 2, respectively, of *Verticillium dahliae* on tomato leaflets

Cultivar	Bioassay symptoms[a]: Race 1 ATR-13[b]	20A[b]	Race 2 50A[b]	Ruhama2[c]	91A[c]
Susceptible					
Hosen Eilon	+	+	+	+	+
Roma F	+	+	+	+	+
Vendor	+	+	+	+	+
Ve gene					
Roma VF	−	−	+	+	+
VF 134-1	−	−	+	+	+
Pakmor	−/+	−	+	+	+
M-82-1-8 VF	−	−	+	+	+
Tropik	−/+	−	+	+	+

[a]The bioassay performed by injection of 100 μl peptide solution per leaflet. Symptoms: (+) = chlorosis and necrosis observed at 18–36 hr following injection; (−/+) = chlorosis only at the injection site; (−) = no visible reaction at 48 hr.
[b]Concentration of 5 $\mu g\ ml^{-1}$ (w/v) in water.
[c]Concentration of 3 $\mu g\ ml^{-1}$ (w/v) in water.

on *Ve* and susceptible tomato plants appears to represent one of the few cases in which single gene disease resistance is associated with tolerance or insensitivity to a pathogen-produced toxic metabolite. Single resistance genes generally condition hypersensitive defense reactions, but our results showing that the *Ve* gene confers a degree of insensitivity to the race 1 peptide indicate that this gene behaves differently. The unique nature of the *Ve* gene is also suggested by the fact that race 1 of *V. dahliae* colonized *Ve* tomato stems despite the absence of disease symptoms (Tables 1 and 2). The bases of the host-specific nature of the race 1 peptide toxin and the tolerance of *Ve* tomato cells to the race 1 but not race 2 peptide are not known. Significantly, however, the tolerance of *Ve* tomato plants to the race 1 peptide seems to have a cellular basis because tolerance was expressed by intact leaflets, excised root tips, and suspension culture cells (Tables 4–6 and Fig. 2).

The toxic peptides prepared from race 1 and 2 isolates of *V. dahliae* showed similar chromatographic behavior during gel filtration (data not shown) and HPLC (Fig. 1) and both appeared to contain 11 amino acids (Table 3). However, they differed in amino acid composition and exhibited different toxic activity on *Ve* tomato plants. The differential pathogenicity of the two *V. dahliae* races may therefore be due to their production of the different peptide toxins. This is similar to the conclusion of Buchner et al (5), who showed that the peptide toxin produced by a pathogenic potato isolate of *V. dahliae* was altered or absent in a spontaneous nonpathogenic mutant strain of the fungus. It is noteworthy in this regard that even small differences in the amino acid composition of biologically active peptides may cause large differences in activity. For example, studies of synthetic analogs of a nonapeptide, porcine serum thymic factor showed that minor changes at the carboxy terminus abrogated biological activity (3). Conversely, the potency of beta-endorphin was increased by replacing a single amino acid (14).

The Verticillium peptide toxins are not as potent as certain host-selective toxins, giving activity at the $\mu g\ ml^{-1}$ range, and the

TABLE 5. Phytotoxicity to tomato root tips of purified peptide toxins isolated from *Verticillium dahliae* races 1 or 2

	Phytotoxic reaction[a]					
	Race 1 (ATR-13)			Race 2 (Ruhama 2)		
Cultivar	$10\ \mu g\ ml^{-1}$	$5\ \mu g\ ml^{-1}$	$1\ \mu g\ ml^{-1}$	$10\ \mu g\ ml^{-1}$	$5\ \mu g\ ml^{-1}$	$1\ \mu g\ ml^{-1}$
Susceptible						
Hosen Eilon	+	+	−	+	+	−
Roma F	+	+	−	+	+	−
Ve gene						
Roma VF	+	−	−	+	+	−
VF 134	+	−	−	+	+	−

[a]+ = Phytotoxicity (little or no fluorescence); − = most cells viable (pronounced fluorescence). Toxin was added to root tips in a depression slide and incubated for 6 hr followed by 1 hr incubation with 0.01% sodium fluorescein.

TABLE 6. Viability of tomato suspension cells following 4 hr incubation with purified peptide toxins from *Verticillium dahliae* races 1 or 2

	Viable cells[a] (%)					
	Race 1 (ATR-13)			Race 2 (Ruhama 2)		
Tomato cultivar	$10\ \mu g\ ml^{-1}$	$5\ \mu g\ ml^{-1}$	$1\ \mu g\ ml^{-1}$	$10\ \mu g\ ml^{-1}$	$5\ \mu g\ ml^{-1}$	$1\ \mu g\ ml^{-1}$
Roma F (*ve*)	0	25	35	0	0	25
Roma VF (*Ve*)	25	90	90	0	0	10

[a]Toxin added to cell suspensions and incubated for 4 hr at room temperature; after an additional 1 hr incubation with 0.01% sodium fluorescein, cells viewed under fluorescence microscope; percentage of viable cells was determined based on counts of at least 100 cells. Positive fluoresence was assumed to denote viable cells (see Fig. 2).

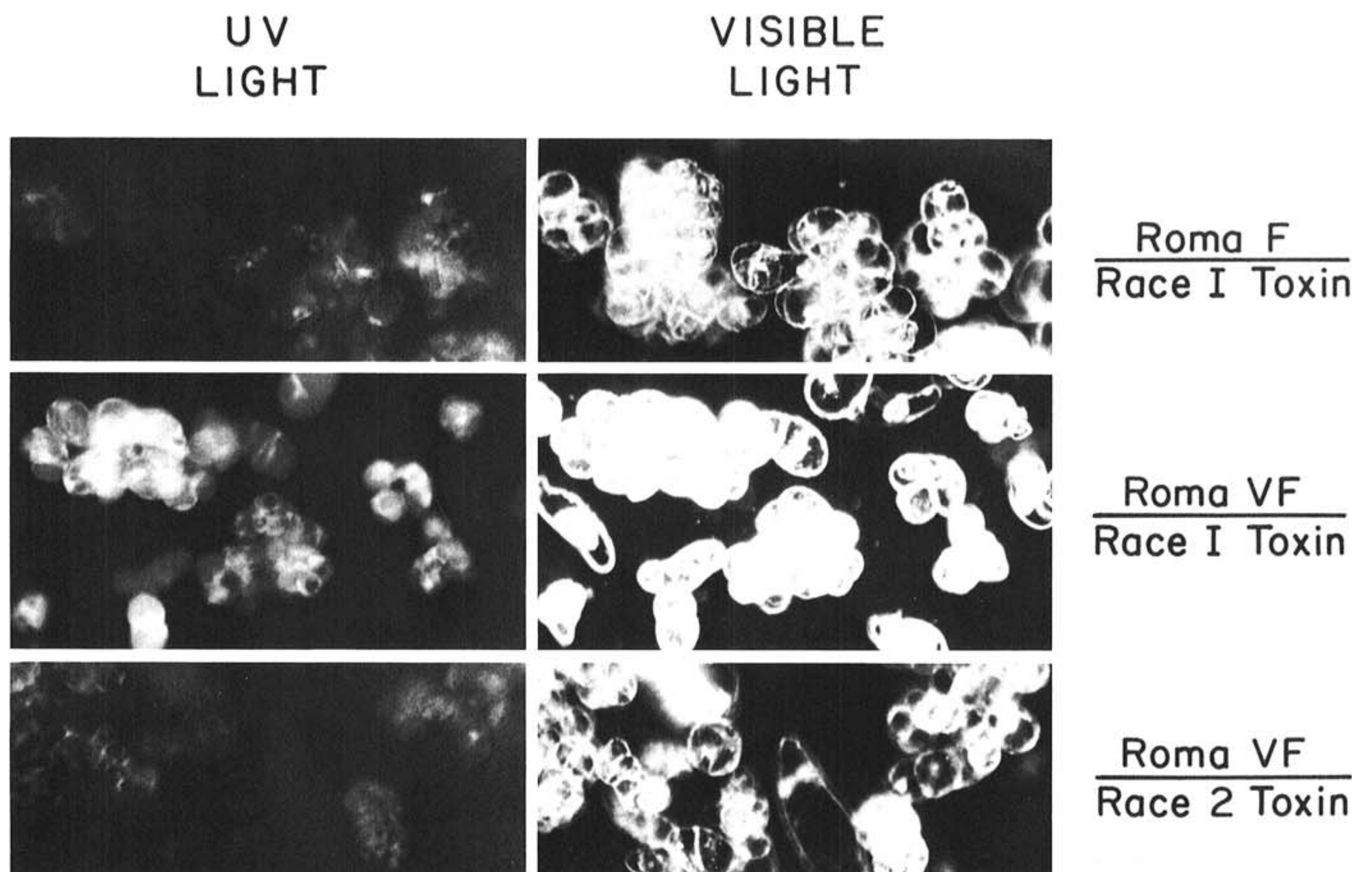

Fig. 2. Effect of purified *Verticillium dahliae* peptide toxins on fluorescein uptake by tomato suspension cells. Peptides were supplied as described in methods at $5\ \mu m\ ml^{-1}$. In all cases, cells were photographed under UV light (left) to visualize fluorescence and under visible light (right). Appearance of pronounced fluorescence was assumed to denote cell viability and lack of fluorescence to denote toxin-incited loss of viability.

difference in response of resistant and susceptible tomato cultivars is only about one order of magnitude (Tables 5 and 6). Despite these differences from the recognized host-selective toxins (19), the differential activity of the race 1 peptide from tomato isolates of *V. dahliae* on tomato cultivars carrying or lacking the *Ve* gene demonstrates that this peptide indeed possesses host-selective properties.

It is as yet unclear whether the *Ve* gene confers resistance to race 1 of *V. dahliae* solely by conferring tolerance to the race 1 peptide toxin or whether the gene is pleiotropic. For instance, several investigators have obtained evidence with tomato and several other plants suggesting that tylose formation and phytoalexin production are greater in resistant than in susceptible plants (11,22). It has also been shown with other diseases that pathogen-produced metabolites can suppress phytoalexin production by the plant (20,21). Further work will be required to determine whether the *Ve* gene functions pleiotropically or whether the reported differential phytoalexin and tylose responses result from their suppression in tomato plants lacking the *Ve* gene.

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. Ashworth, L. J., Jr., Huisman, O. C., Harper, D. M., and Stromberg, L. K. 1979. Verticillium wilt disease of tomato; influence of inoculum density and root extension upon disease severity. Phytopathology 69:490-492.
3. Bach, J. F., Bach, M. A., Dardenne, M., Pleau, J. M., Lefrancier, P., Choay, J., Blanot, D., and Bricas, E. 1980. Serum Thymic Factor: A Peptide Lymphocyte-Differentiating Hormone. Pages 489-499 in: Polypeptide Hormones. R. F. Beers, Jr. and E. G. Bassett, eds. Raven Press, New York.
4. Bender, C. G., and Shoemaker, P. B. 1984. Prevalence of Verticillium wilt of tomato and virulence of *Verticillium dahliae* race 1 and race 2 isolated in Western North Carolina. Plant Dis. 68:305-309.
5. Buchner, V., Nachmias, A., and Burstein, Y. 1982. Isolation and partial characterization of a phytotoxic glycopeptide from a protein-lipopolysaccharide complex produced by a potato isolate of *Verticillium dahliae*. FEBS Lett. 138:261-264.
6. Conover, R. A. 1959. Verticillium wilt of tomato in Dade County, Florida. Proc. Fla. State Hortic. Soc. 72:199-201.
7. Gamborg, O. L., and Wetter, L. P. 1975. Plant Tissue Culture Methods. National Research Council of Canada, Saskatoon. 110 pp.
8. 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.
9. Hall, D. H., and Kimble, K. A. 1972. An isolate of *Verticillium* found pathogenic to wilt-resistant tomatoes. Calif. Agric. 26:3.
10. Hare, P. E. 1977. Subnanamole-range amino acid analysis. Meth. Enzymol. 47:3-18.
11. Harrison, N. A., and Beckman, C. H. 1982. Time/space relationships of colonization and host response in wilt-susceptible cotton (*Gossypium*) cultivars inoculated with *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum*. Physiol. Plant Pathol. 21:193-207.
12. Jones, J. P., and Crill, P. 1975. Reaction of resistant, tolerant and susceptible tomato varieties to Verticillium wilt. Plant Dis. Rep. 59:3-6.
13. Keen, N. T., Long, M., and Erwin, D. C. 1972. Possible involvement of a pathogen-produced protein-lipopolysaccharide complex in Verticillium wilt of cotton. Physiol. Plant Pathol. 2:317-331.
14. Li, C. H., Yamashiro, D., and Nicolas, P. 1982. Beta-endorphin: Replacement of tyrosine in position 27 by tryptophan increases analgesic potency—preparation and properties of the 2-nitrophenyl-sulfenyl derivative. Proc. Nat. Acad. Sci. (USA) 79:1042-1044.
15. Nachmias, A., Buchner, V., and Krikun, J. 1982. Comparison of protein-lipopolysaccharide complexes produced by pathogenic and non-pathogenic strains of *Verticillium dahliae* Kleb. from potato. Physiol. Plant Pathol. 20:213-221.
16. Nachmias, A., Buchner, V., and Burstein, Y. 1985. Biological and immunochemical characterization of a low molecular weight phytotoxin isolated from a protein-lipopolysaccharide complex produced by a potato isolate of *Verticillium dahliae* Kleb. Physiol. Plant Pathol. 26:43-55.
17. Robinson, D. E., Larson, R. H., and Walker, J. C. 1957. Verticillium wilt of potato in relation to symptoms, epidemiology and variability of the pathogen. Wisc. Agric. Exp. Stn. Res. Bull. 202. 49 pp.
18. Schaible, L., Cannon, O. S., and Waddoups, V. 1951. Inheritance of resistance to *Verticillium* wilt in a tomato cross. Phytopathology 41:986-990.
19. Scheffer, R. P., and Livingstone, R. S. 1984. Host-selective toxins and their role in plant diseases. Science 223:17-21.
20. Shiraishi, T., Oku, H., Yamashita, M., and Ouchi, S. 1978. Elicitor and suppressor of pisatin induction in spore germination fluid of pea pathogen, *Mycosphaerella pinodes*. Ann. Phytopathol. Soc. Jpn. 44:659-665.
21. Tietjen, K., and Matern, U. 1984. Induction and suppression of phytoalexin biosynthesis in cultured cells of sunflower, *Carthamus tinctorius*, by metabolites of *Alternaria carthami*. Arch. Biochem. Biophys. 229:136-144.
22. Tjamos, E. C., and Smith, I. M. 1975. The expression of resistance to *Verticillium albo-atrum* in monogenically resistant tomato varieties. Physiol. Plant Pathol. 6:215-225.
23. Wilhelm, S. 1975. Sources and nature of Verticillium wilt resistance in some major crops. Pages 166-171 in: Biology and Control of Soil-Borne Plant Pathogens. G. W. Bruehl, ed. American Phytopathological Society, St. Paul, MN.