Antifungal Activity of Extracts from Fusarium Wilt-Susceptible and -Resistant Tomato Plants

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Appreciation is expressed to Dr. E. H. Allen for his suggestions and encouragement and to E. J. Koch, Biometrical Services, for statistical analysis.

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ABSTRACT

Antifungal activity of extracts from resistant tomato roots was almost twice as great as that from susceptible tomato roots, whether or not the plants were infected with *Fusarium oxysporum* f. sp. *lycopersici*. Presumptive chromatographic and bioassay data suggests that the fungitoxicity of the extracts is caused by α-tomatine. The amount of α-tomatine per gram fresh weight of infected wilt-resistant plants was estimated as 180 μg.

Phytopathology 65:93-94

Additional key words: paper bioassay.

The possibility that host fungitoxicants account for varietal resistance of tomato plants to Fusarium wilt has been explored by many researchers. The toxicity of crude saps (2) and α-tomatine (3, 6) have been extensively investigated for their roles in resistance.

We found the amount of fungitoxic activity in roots of our healthy resistant tomato to be significantly greater than that in the healthy susceptible variety. The objective of this study was to determine whether these differences in levels of fungitoxicant in the roots still exist when resistant and susceptible tomato varieties are wounded or infected with *Fusarium*.

MATERIALS AND METHODS.—Two cultivars of tomato, *Lycopersicon esculentum* Mill. [wilt-resistant Improved Pearson (IP), and wilt-resistant Pearson VFII (VF)] were used. The plants were grown under greenhouse conditions at 23-27 C. Normally, plants were 15-21 days old when inoculated. Other materials and cultural methods for host and pathogen have been previously described (7).

Tomato tap roots were severed 1 mm from the root tip, washed in sterilized distilled water, and the plants were inoculated by placing the root system in a spore suspension of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hanson race 1. Both inoculated plants and wounded noninoculated control plants were repotted and kept in the greenhouse.

Root sections were harvested 6 days after inoculation and homogenized with methanol (30 ml/g fresh tissue) in a blender. The homogenate was filtered through Whatman No. 3 filter paper, and the filtrate was evaporated to 0.1 ml in a rotary evaporator at 30 C. The residue was resuspended in 40% methanol and kept at −18 C overnight. The methanol extract was centrifuged at 5,000 *g* for 5 minutes. The supernatant liquid was evaporated to 0.1 ml, diluted with 10ml distilled water, and adjusted to pH 4 with 1 N HCl. The aqueous solution was shaken three times with 30 ml diethyl ether (freshly distilled) and the ether phase was discarded. The aqueous phase was evaporated to remove traces of ether. Enough methanol was added to make a final volume of 1 ml/g fresh tissue.

Mycelial growth test.—Solutions used to detect antifungal activity in extracts (PDB bioassay) were prepared by evaporating 4 ml of extract and 2 ml of potato-dextrose broth (PDB) to dryness (9). The residue was resuspended in 2 ml sterilized distilled water, and adjusted to pH 4.5. The assay organism, *F. oxysporum* f. sp. *lycopersici,* race 1, was cultured on potato-dextrose agar in petri dishes for 6 days at 28 C. Five-mm-diameter plugs of mycelium were cut from the petri dishes and placed in 2 ml of test solution in Stender dishes. Radial growth of the mycelium was measured after 18 hours at 28 C in the dark.

A paper bioassay (PBA) described by Allen and Kuć (1) was used to determine the presence of α-tomatine in extracts. Test solutions were spotted on Whatman No. 3 MM paper and the papers developed in (A) 5% acetic acid and (B) *n*-butanol:acetic acid:water (4:1:1, v/v). The chromatograms were air-dried for at least 24 hours and then placed in Pyrex dishes on two layers of cheesecloth supported on glass racks covered with wire screening. The chromatograms were sprayed with a pH 4.5 suspension of conidia of *Helminthosporium carbonum* Ullstrup, which had been cultured on V8 agar at 24 C. Warm water was placed under the rack containing the chromatogram. The Pyrex dishes with the seeded chromatograms were incubated at 28 C. After 24-36 hours, the chromatograms were examined for inhibitory spots, which appeared as white areas on a black background of germinated spores.

RESULTS.—Growth in extracts of wounded roots did not differ from growth in extracts of infected roots of the same cultivar (Table 1). Mycelial growth in extracts of susceptible roots was almost twice as much as growth in extracts of resistant root tissue, whether or not the plants were infected with *F. oxysporum*.

Extracts prepared from 0.5 g infected VF plants and 120 μg pure α-tomatine (ICN - K & K Laboratories, Plainview, NY 11803) were spotted singly and also as a combined sample and co-chromatographed. The chromatograms were developed in solvents A and B, and bioassayed by PBA. The R_f values for extract and α-tomatine in solvent A were 0.36 and 0.43, respectively; and in solvent B were 0.77 and 0.80, respectively. The R_f values for pooled extract and α-tomatine were 0.74 in solvent A and 0.3 in solvent B. The chromatographic mobilities of extract and α-tomatine overlapped when the compounds were spotted separately, but were identical when spotted together. This suggests that the fungitoxicity in extracts was caused by α-tomatine. When spotted alongside several amounts of α-tomatine, extract from 2 g infected root tissue yielded a zone of inhibition

TABLE 1. Effect of methanol extracts of tomato roots on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*, race 1

Tomato cultivar	Growth of *Fusarium* in PDB containing extracts from tomato (%)	
	Wounded, not inoculated	Wounded, inoculated
Pearson VF11 (resistant)	37.63 B[a]	38.00 B
Improved Pearson (susceptible)	64.00 A	58.88 A

[a]Each value represents the mean of eight observations. Means not followed by the same letter are significantly different, $P = 0.01$, according to Duncan's multiple-range test. Data represent mycelial extension of *F. oxysporum* in potato-dextrose broth (PDB) containing tomato extracts as a percentage of mycelial extension in PDB alone.

equivalent to 360 μg α-tomatine. The amount of α-tomatine in PDB required to reduce mycelial growth of *F. oxysporum* 50% was 550 μg. Percentage of growth in 360 μg α-tomatine and in extract of 2 g infected VF root tissue (Table 1) was 37%.

DISCUSSION.—Because the fungitoxicity of the extracts of our two cultivars was significantly different, we concluded that α-tomatine could play a role in resistance. Our results conflict with those of Langcake et al. (6), who concluded that tomatine was not involved in the resistance of tomato to *Fusarium*, after finding that tomatine concentration increased in the roots of infected plants of both resistant and susceptible varieties. The difference between the two results could be ascribed to varietal differences (4, 6, 8) or to differences in the age of the plants used (5, 6, 8).

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