Virulence of *Verticillium dahliae* and *V. albo-atrum* Isolates in Tomato Seedlings in Relation to Their Host of Origin and the Applied Cropping System

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ABSTRACT

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The pathogenic variation of 334 *Verticillium dahliae* and *V. albo-atrum* isolates in tomato seedlings was investigated. Disease severity assessed by symptom development and host colonization, showed that strains isolated from 27 plant species with a widespread distribution in Greece, varied from nonpathogenic to highly virulent on the susceptible tomato cultivar Early Pak. The degree of pathogenicity to tomato was seldom related to the plant species from which the isolate was obtained, but was dependent on the previous cropping history. Isolates obtained following monoculture of nonsolanaceous hosts were nonpathogenic or mildly pathogenic while isolates originating from areas of a diversified cropping system, including tomatoes and other vegetables, generally were highly pathogenic to tomato.

Variation in pathogenicity of *Verticillium* species to cultivated plants has frequently been demonstrated. Early reports (8) showed that *Verticillium dahliae* isolates from different host plants (*Aconitum napellus, Anthirinum* sp., *Lathyrus odoratus, Papaver rhoeas, Rhus canadensis, Rubus idaeus, Ulmus* sp., apricot, cherry, cucumber, melon, and potato) are pathogenic to tomatoes. Some reports indicated that *V. dahliae* isolates from strawberries (1), okra (11), safflower (15), tomato, eggplant, pepper (5,14), potato, apricot, peach (14), and cantaloupe (10,14) are also pathogenic to tomatoes. Other reports (2–4,10,12) indicated that isolates of *V. dahliae* from artichoke, peppermint, olive and cotton are not pathogenic on tomatoes. Some cotton isolates of *V. dahliae* that were mildly virulent on cotton cultivar Acala 4-42 were pathogenic to tomato, but isolates highly virulent on cotton were essentially nonpathogenic to tomato (9). *V. dahliae* isolates from olive were reported to be pathogenic to tomatoes (5).

The purpose of the present work was to determine whether the variation in pathogenicity to tomato of *V. dahliae* and *V. albo-atrum* isolates is related to the host species from which the fungus was obtained, and whether there was a relationship between virulence and the previous cropping history.

MATERIALS AND METHODS

A collection of 306 isolates of *V. dahliae* (microsclerotial cultures) and 28 of *V. albo-atrum* (dark mycelial) isolates obtained from diseased plant specimens was tested for virulence on tomato seedlings. Infected plant material was obtained through specimens that were brought to Benaki Phytopathological Institute or collected by the author during field surveys in Greece between 1974 and 1979. The isolates originated from plants belonging to 12 families. All isolates were cultured on potato dextrose agar (PDA). Isolates forming no resting structures were discarded. Inoculum for plates was prepared by homogenizing 2 cm^3 of a PDA culture in 20 ml distilled sterile water for 30 sec in a Sorvall Omnimixer. Five to six milliliters of the homogenate were uniformly spread over the PDA surface of each of four petri dishes. Plates were incubated for 7–10 days at 22 C. Inoculum was prepared by blending the contents of four plates for 1 min in 200 ml, and then diluted up to a total volume of 800 ml of distilled sterile water. Final inoculum concentration averaged 8×10^6–10^7 viable propagules per milliliter (mixture of mycelia, microsclerotia, and conidia) as determined by dilution plate counts on PDA. Forty tomato seedlings of the susceptible cultivar Early Pak and 40 of the resistant cultivar Ace 35 VF were inoculated at the stage of the appearance of the first true leaf. The cultivars Precoce, Craigella, and Super Marmande (all lacking the *Ve* gene) also were occasionally used in addition to Early Pak and Ace 35 VF. The procedure was a modification of the technique described by Retig et al (7). The seedlings were transferred to plastic containers (800 ml capacity, 15 × 10 × 6 cm dimensions) containing 25% Hoagland's nutrient solution. Groups of five seedlings were supported by eight holes in a plastic lid. Culture homogenate (400 ml for each cultivar) replaced the nutrient solution for 48 hr. After the removal of the inoculum, 25% Hoagland's solution was again added and the treated plants were kept in a glasshouse under controlled light and temperature conditions (12–14 hr daily

TABLE 1. Virulence of isolates of *Verticillium dahliae* and *V. albo-atrum* in susceptible tomato seedlings[a] in relation to the plant families from which they originated

Plant family of origin	Total number of isolates tested	Disease index 0–1.99[b]	2–2.99	3–3.99	4–5
Anacardiaceae	13	6	3	3	1
Compositae	12	11	1	...	...
Cucurbitaceae	6 (7)[c]	4 (2)	··· (2)	2 (2)	··· (1)
Malvaceae	63	57	1	5	...
Oleaceae	83	37	12	25	9
Rosaceae	28	22	2	2	2
Solanaceae	92 (21)	17 (1)	20 (4)	39 (14)	16 (2)
Vitaceae	4	3	...	1	...
Leguminosae	1	...	...	1	...
Labiatae	1	1	...	...	...
Caprifoliaceae	1	...	...	...	1
Lauraceae	2	2	...	...	...
Totals	306 (28)	160 (3)	39 (6)	78 (16)	29 (3)

[a] Data refer to the susceptible tomato cultivar Early Pak. Several isolates tested on other tomato cultivars also lacking the *Ve* gene for resistance gave similar results to those on Early Pak.

[b] Disease severity rated from 0–1.99; 2.00–2.99; 3.00–3.99; and 4.00–5.00 respectively. Average disease index rating in 40 tomato seedlings per isolate 30 days after inoculation. Rating based on a scale of 0–5; 0 = no colonization, no external symptoms, plant healthy; 1 = no colonization, cotyledons dead; 2 = colonization detected, cotyledons dead; 3 = as 2 plus flaccidity, chlorosis and/or necrosis in the first pair of leaves; 4 = as 3 plus flaccidity, chlorosis and/or necrosis in the second pair of leaves; 5 = lethal reaction.

[c] Numbers within parentheses refer to isolates of *V. albo-atrum*.

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photoperiod at 19–22 C). The inoculated plants were transferred at the beginning of the light phase of the photoperiod. Disease severity was based on symptom development in 40 seedlings 30 days after inoculation (Table 1). Vessel colonization was checked by plating a small piece of first internode stem from each seedling onto plates of a selective medium (1.5 g plain agar, 10 mg streptomycin, 20 mg penicillin (500,000 units of each) and 1 ml of absolute alcohol in 200 ml of distilled sterile water) and examining them 10 days later for the presence of mycelium or resting structures.

RESULTS

Inoculated seedlings of the susceptible tomato cultivars developed symptoms slowly when infected with mildly pathogenic and rapidly with highly pathogenic isolates. Flaccidity of cotyledons appeared 5–7 days after inoculation with some isolates. Symptoms including leaf flaccidity, chlorosis, desiccation or leaf drop were seen with the progress of the disease. Highly pathogenic and extremely pathogenic isolates affected 100% of the seedlings within 25–30 days after inoculation.

Only one of the isolates caused symptoms on the seedlings of the Ace 35 VF. The isolate of *V. dahliae* highly pathogenic to both Early Pak and Ace 35 VF tomatoes originated from a diseased eggplant. This is the first record of the occurrence of race 2 of *V. dahliae* in Greece.

The disease ratings showed that isolates from Greece of *V. dahliae* and *V. albo-atrum* vary considerably in virulence on Verticillium-susceptible seedlings (Table 1). Of the 334 isolates tested 163 were given a disease severity index of 0–1.99, 45 were rated 2–2.99, 94 were rated 3–3.99 and 32 were rated 4–5. Variation in pathogenicity was observed among isolates obtained from diseased plants belonging to the same botanical species. For instance, the 13 isolates of *V. dahliae* from pistachio trees (Anacardiaceae) varied from nonpathogenic to very pathogenic in tomato seedlings. Variation in pathogenicity of isolates from a given host plant was a common phenomenon.

Ninety-two *V. dahliae* and 21 *V. albo-atrum* isolates from solanaceous hosts were generally pathogenic to the susceptible tomato cultivars with minor exceptions. Finally, few *V. dahliae* isolates obtained from vine, ivy, judas tree, peppermint, lauristinus, and laurel showed variation in their pathogenicity to tomato.

The results for individual isolates suggested a relationship between virulence in tomato and the previous crops grown in a particular region (Table 2). There was a higher frequency of nonpathogenic isolates from nonsolanaceous hosts cultivated as a monoculture (ie, pistachio, olive, watermelon, rose, almond, and okra). Isolates from the same host species that originated from areas where solanaceous plants also are extensively grown tended to be more virulent in tomato. Most isolates from cotton, artichoke, and peach were nonpathogenic to mildly pathogenic in tomato. These isolates were obtained from fields in which tomatoes had not been grown, grown on a limited basis, or recently

TABLE 2. Numbers of isolates of *Verticillium dahliae* and *V. albo-atrum* of different levels of virulence on susceptible tomato seedlings[a] in relation to their host of origin and the cropping system

		Disease index							
		Single cropping system[b]				Diversified cropping system[c]			
Host of origin	Total number	0–1.99	2–2.99	3–3.99	4–5	0–1.99	2–2.99	3–3.99	4–5
Anacardiaceae									
Pistachio tree	13	5	1	...	...	1	2	3	1
Compositae									
Artichoke	7	7	...	...	...	...	...	...	...
Cockle bur	1	1	...	...	...	...	...	...	...
Dahlia	2	2	...	...	...	...	...	...	...
Gerbera	1	...	...	...	...	...	1	...	...
Marigold	1	1	...	...	...	...	...	...	...
Cucurbitaceae									
Cucumber	(7)[d]	...	...	...	...	(2)	(2)	(2)	(1)
Melon	2	2	...	...	...	...	...	...	...
Watermelon	4	2	...	...	...	...	...	2	...
Malvaceae									
Cotton	60	55	...	...	...	...	1	4	...
Okra	3	2	...	...	...	...	...	1	...
Oleaceae									
Olive tree	83	28	3	1	...	9	9	24	9
Rosaceae									
Almond tree	9	8	...	...	...	...	...	1	...
Apricot tree	1	...	...	...	...	...	1	...	...
Peach tree	11	11	...	...	...	...	...	...	...
Rose	5	2	...	...	...	1	...	1	1
Strawberry	2	...	...	...	...	...	1	...	1
Solanaceae									
Eggplant	17	...	...	...	...	4	3	6	4
Green pepper	5	...	...	...	...	2	1	2	...
Potato	12 (20)	...	...	...	...	6 (1)	3 (4)	2 (13)	1 (2)
Tomato	58 (1)	...	...	...	...	5	13	29 (1)	11
Vitaceae									
Ivy	3	3	...	...	...	...	...	...	...
Vine	1	...	...	...	...	...	...	1	...
Various families									
Judas tree	1	...	...	...	...	...	...	1	...
Peppermint	1	1	...	...	...	...	...	...	...
Lauristinus	1	...	...	...	...	...	...	1	...
Laurel	2	2	...	...	...	...	...	...	...
Totals	306 (28)	132	4	1	...	28 (3)	35 (6)	78 (16)	28 (3)

[a]Data refer to the susceptible tomato cultivar Early Pak. See Table 1 for explanation of categories of virulence.
[b]Cultivation of one or few Verticillium-susceptible host plants in the absence of tomatoes or other solanaceous species.
[c]Cultivation of several Verticillium-susceptible host plants including tomatoes and/or solanaceous species.
[d]Numbers within parentheses refer to *V. albo-atrum* isolates.

introduced. One isolate from cotton from Helia County and four from Preveza County, where both cotton and tomatoes had been grown, were found to be highly pathogenic in tomatoes. Isolates of *V. dahliae* and *V. albo-atrum* from potato that originated from the highlands, where potatoes are cultivated for seed production, were pathogenic to tomato regardless of the diversity of the previous crops grown.

DISCUSSION

The current work has clearly shown that isolates of *V. dahliae* and *V. albo-atrum* in Greece vary in virulence to tomatoes. This variation was only rarely related to the host species from which the isolate originated. It has also been reported (6) that peppermint isolates that were initially avirulent to tomato became virulent after successive passages through a susceptible tomato cultivar. This broadening of host range could take place in the field after the introduction of tomato cultivation. This might have happened in several districts where cotton has been extensively grown in Greece. All *V. dahliae* isolates obtained from Boeotia County, where cotton is the main crop, were nonpathogenic to the tomato cultivar Early Pak. However, it was found that isolates obtained from diseased tomatoes recently cultivated in the same area were virulent to the same cultivar. This could imply: introduction of new isolates into the area, selection of formerly existing (but rare) isolates of high pathogenicity to tomato, or adaptation of *V. dahliae* strains to a new host possibly after repeated crops in fields previously planted to cotton. On the other hand, isolates from cotton from areas (Helia, Preveza) where solanaceous plants are also grown exhibited a high degree of pathogenicity to tomato. The absence of specialization of these isolates was also documented. Isolates from almond, pistachio, olive, rose, okra, and watermelon were of low virulence when obtained from areas of monoculture, but some that originated from hosts growing in a multicrop system were virulent. The possibility, however, that isolates obtained from areas with a diversified cropping system may be mixtures of genotypes or "pathotypes" cannot be excluded. Vigouroux (13) stated that isolates obtained from a monoculture are of low pathogenicity to hosts other than the one from which they have originated. He also claimed that Verticillium-susceptible crops prevailing in a particular region constitute a determinative factor for natural selection of the quantitative and qualitative traits in *Verticillium* populations in cultivated soils. The present study seems to support his concepts of selection. In the present study, monoculture of nonsolanaceous hosts seems to favor strains nonpathogenic to tomato, and a mixed cropping system, including solanaceous hosts, seems to favor virulent strains. The evidence, however, is indirect because the fungus was not isolated from the soil, but only from the diseased plants.

Some practical benefits may be derived from the work reported in this paper. For example, if even a small fraction of the isolates present in the field after several years of cotton cultivation are pathogenic to tomato, then crop rotation systems including tomatoes might not preclude the occurrence of Verticillium wilt of tomato.

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