Verticillium Wilt of Paprika Caused by a Highly Virulent Isolate of *Verticillium dahliae*

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ABSTRACT

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Verticillium dahliae caused wilting, stunting and early dying of paprika plants in Israel, resulting in a 22% reduction in yield. A *V. dahliae* isolate from diseased paprika plants was slower growing on agar medium than *V. dahliae* isolates from potato, tomato, watermelon, and *Dodonaea*, but formed microsclerotia earlier. The paprika isolate of *V. dahliae* was highly virulent to paprika in pathogenicity tests. Disease severity indices obtained from three paprika cultivars ranged from 3.7 to 4.9 (on a scale of 0 to 5). The disease caused height reductions of 43 to 62% in the three inoculated paprika cultivars, and pathogen colonization levels were 10- to 35-fold higher in stems than in leaves. The potato isolate of *V. dahliae* did not produce symptoms or cause stunting of paprika, and could not be detected in stems or leaves. The tomato, watermelon, and *Dodonaea* isolates did not cause disease symptoms in paprika, although they colonized the plant tissue. In potato and eggplant, the paprika isolate caused a higher incidence of disease with more severe symptoms and a higher level of tissue colonization than did the potato isolate.

Additional keywords: eggplant, pathogenicity, potato, yield reduction

Paprika (*Capsicum annuum* L.) is grown mainly in the Negev region in the southern part of Israel, and its production has increased 100-fold (to about 400 ha) during the last 15 years. Paprika is grown in rotation with potato, despite the prevalence of the soilborne fungus *Verticillium dahliae* Kleb. (7,10), and as the crop had not displayed symptoms it was not considered susceptible to *V. dahliae* in Israel. However, wilt in green pepper caused by *V. dahliae* had been reported once from Israel (8). In Morocco, where pepper is grown in rotation with tomato, a wilt of pepper caused by *V. dahliae* has been recently reported (3). *V. dahliae* has been isolated from diseased plants of *Capsicum* spp. in other parts of the world. These isolates were reported to cause severe disease symptoms, stunting and wilting, when inoculated on different crops such as tomato and eggplant (5,11,14).

Recently, paprika plants (cv. Admoni) grown in the Negev region wilted, collapsed, and desiccated before harvest. Discoloration of the vascular bundles was observed and microsclerotia were present on roots. The disease occurred in several large patches in the field, covering 1 to 5

ha, and resulted in yield losses of 22% (reduction of 1.5 ton of dry matter per hectare).

The objective of this study was to identify and characterize the causal agent of wilting and death in paprika.

MATERIALS AND METHODS

Plant material. Paprika seeds of cultivars Shalhevet, Lehava, and Admoni, and eggplant seeds (cv. Black Beauty), were disinfested with sodium hypochlorite (0.3% for 3 min.) and grown in trays containing a sterile mixture of peat, perlite, and vermiculite (1:1:1) for 3 to 4 weeks (until 3 true leaves developed). Potato plantlets were grown for 3 to 4 weeks from sprouts cut from certified surface-sterilized (0.3% HClO) seed tubers of the *V. dahliae* susceptible cv. Nicola, until 3 leaves expanded.

Source of *V. dahliae* isolates. *V. dahliae* isolates from paprika (Vds), potato (Vdp), tomato (Vdt), watermelon (Vdw), and *Dodonaea* (Vdd) were used in this study. The isolates were obtained by placing 5-mm surface-sterilized stem segments from diseased host plants (from commercial production) on potato dextrose agar (PDA; Difco Laboratories, Detroit) supplemented with 100 ppm streptomycin sulfate. Plates were incubated in the dark at 27°C for 10 days. Selected colonies were subcultured to PDA without antibiotics.

Morphology of isolates. Discs (5-mm-diameter) were taken from 4-day-old cultures and plated on PDA in 90-mm-diameter petri dishes. The plates were in-

cubated in the dark at 27°C and the diameter (cm) of resulting colonies was measured. The appearance of microsclerotia and their eventual distribution in the colony and the extent of aerial mycelium were recorded. There were 16 replicated plates for each isolate.

Inoculation of plants. *V. dahliae* was cultured in potato dextrose broth (PDB, Difco Laboratories) at 27°C for 3 to 4 days with continuous shaking (110 rpm). The culture was filtered through four layers of muslin gauze to separate mycelium and conidia. The concentration of the conidia, as determined by serial dilution and plating on PDA, were adjusted with sterile distilled water to 2 to 6×10^6 CFU/ml.

Twenty seedlings of each paprika cultivar, as well as eggplant and potato plantlets, were inoculated with Vds and Vdp isolates by immersing their roots in a conidial suspension for 20 min. In a separate experiment, 20 pepper seedlings (cv. Lehava) were similarly inoculated with Vds, Vdt, Vdw, and Vdd isolates. Control seedlings were dipped in sterile distilled water. Seedlings were transferred to pots containing a sterile mixture of peat, vermiculite, and sand and grown in a growth chamber (22 to 25°C, 12 h light, 100 μ En⁻²s⁻¹). Experiments were repeated three times.

Disease evaluation. Disease incidence was determined as percentage of plants displaying symptoms 26, 31, and 49 days after inoculation for paprika; 10 days after inoculation for potatoes; and 17 days after inoculation for eggplants. Symptom severity was scored on a scale of 0 to 5, where 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = moderate (30 to 50% of leaves) wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe (> 50% of leaves) wilt and necrosis, and 5 = death of plant.

Colonization of plant tissue by *V. dahliae* was determined by placing 8 to 12 segments from the base (1 to 4 cm above the crown), middle (2 to 5 cm), and top (5-cm apex) of each stem on PDA. After 10 days of incubation at 27°C, segments from which *V. dahliae* grew were counted and their percentage of the total was calculated.

The number of CFU/g also was determined from inoculated plants. Ten to twelve surface-sterilized segments (each 20 mm long) cut from the stem base or 8 leaf discs (50-mm diameter) from each plant (the fourth leaf from the apex) were

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macerated in an Ultraturrax blender (Janke Kunkel, Staufen, Germany) with 3 ml sterile water for 60 to 90 s at 4°C. Serial dilutions were prepared and 0.1-ml duplicate aliquots plated on PDA (supplemented with 100 ppm streptomycin sulfate). Plates were incubated for 10 days at 27°C (16) and *V. dahliae* colonies were counted.

Statistical analysis. Data taken as percentages were arcsin-transformed before analysis. Analyses of variance (ANOVA) followed by mean separation using Duncan's multiple range test were done at a significance level of $P = 0.05$.

RESULTS

Growth in culture. After 6 to 8 days, colony diameters of *V. dahliae* isolates Vdp, Vdd, Vdt, and Vdw were signifi-

cantly larger than that of Vds; 21.9, 25.2, 17.9, 16.6 and 14.5 mm, respectively ($P < 0.05$). The Vds isolate did not produce aerial mycelium and the microsclerotia completely covered the colony 6 days after subculturing. The Vdt produced extensive aerial mycelium and the microsclerotia covered 80% of the colony 6 days after subculturing. The other isolates produced little or no aerial mycelium and microsclerotia covered less than 25% of the colony.

Pathogenicity of isolates to paprika. All three paprika cultivars were highly susceptible to Vds. Twenty-six days after inoculation, >80% of plants displayed leaf chlorosis (Table 1). Forty-nine days following inoculation, > 90% of the plants wilted, with mean symptom severity indexes of 4.9, 4.6 and 3.7 in cvs. Admoni,

Lehava, and Shalhevet, respectively. In contrast, no symptoms developed on Vdp-inoculated plants days after inoculation; however, at 49 days, mild symptoms of non-specific leaf chlorosis were observed in Lehava and Admoni (Table 1).

All of the paprika plants inoculated with Vds were stunted. At 26 days the average heights of cvs. Shalhevet, Lehava, and Admoni inoculated with Vds were reduced by 43.6, 62.1, and 43.2%, respectively, compared with the control (Table 1). In contrast, Vdp caused a significant growth response of 31.8% in cv. Lehava at 26 days. However, 49 days after inoculation with Vdp, the heights of all three cultivars were similar to the control plants (Table 1).

In a separate experiment, all Lehava plants inoculated with Vds displayed dis-

Table 1. Disease incidence, disease severity, and height of paprika plants inoculated with *Verticillium dahliae* from paprika (Vds) and potato (Vdp), 26 and 49 days following inoculation

Cultivar	<i>V. dahliae</i> isolate	26 days after inoculation			49 days after inoculation		
		Disease incidence (%) ^x	Disease severity (0-5) ^y	Plant height (cm)	Disease incidence (%)	Disease severity (0-5)	Plant height (cm)
Shalhevet	Vds	85 a ^z	2.3 a	6.0 b	90 a	3.7 a	6.4 b
	Vdp	0 b	0 b	10.4 a	0 b	0 b	14.8 a
	Control	0 b	0 b	10.7 a	0 b	0 b	12.0 a
Lehava	Vds	100 a	2.9 a	5.5 c	100 a	4.6 a	4.7 b
	Vdp	0 b	0 b	14.5 a	3 b	0.7 b	13.2 a
	Control	0 b	0 b	9.9 b	0 b	0 b	10.6 a
Admoni	Vds	80 a	1.0 a	5.9 b	95 a	4.9 a	7.3 b
	Vdp	0 b	0 b	9.7 a	3 b	0.3 b	14.6 a
	Control	0 b	0 b	10.5 a	0 b	0 b	11.2 a

^x Disease incidence based on percentage of plants displaying symptoms.

^y Disease severity rated on a scale of 0 to 5, where 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = moderate wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe wilt and necrosis, and 5 = death of plant.

^z Means within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Table 2. Disease severity, shoot and root weight, height, and tissue colonization of paprika (cv. Lehava) inoculated with *Verticillium dahliae* isolates, 31 days after inoculation

<i>V. dahliae</i> isolate ^x	Disease severity ^y (0-5)	Plant height (cm)	Shoot weight (g)	Root weight (g)	Colonization of root crown (CFU/g)	Colonized stem segments (%)		
						Base	Middle	Top
Vds	2.8 a ^z	5.7 b	1.0 c	0.5 c	2.4×10^4 a	100.0 a	100.0 a	100.0 a
Vdt	0.0 b	10.5 a	5.9 ab	1.3 ab	1.9×10^2 b	80.0 b	63.3 b	6.7 bc
Vdw	0.0 b	10.7 a	6.6 a	1.2 ab	5.1 c	31.7 c	11.7 c	0.0 c
Vdd	0.0 b	10.4 a	5.7 b	0.8 bc	5.1×10^2 b	71.7 b	65.0 b	15.0 b
Control	0.0 b	11.7 a	6.8 a	1.5 a	0.0 c	0.0 d	0.0 c	0.0 c

^x *Verticillium dahliae* isolates; Vds from paprika, Vdt from tomato, Vdw from watermelon, and Vdd from *Dodonaea*; control plants were dipped in sterile distilled water for 20 min.

^y Disease severity rated on a scale of 0 to 5, where 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = moderate (30 to 50% of leaves) wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe (> 50% of leaves) wilt and necrosis, and 5 = death of plant.

^z Means within a column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$).

Table 3. Disease incidence, severity, and tissue colonization of potato and eggplant inoculated with *Verticillium dahliae* from paprika (Vds) and potato (Vdp)

<i>V. dahliae</i> isolate	Potato (10 days after inoculation)			Eggplant (17 days after inoculation)		
	Incidence (%) ^x	Symptoms (0-5) ^y	Colonized stem segments (%)	Incidence (%)	Symptoms (0-5)	Colonized root crown (CFU/g)
Vds	95.0 a ^z	4.7 a	71.9 a	100.0 a	2.67 a	6.4×10^3 a
Vdp	80.0 a	4.0 a	34.0 b	100.0 a	2.33 a	1.5×10^3 b
Control	0.0 b	0.0 b	0.0 c	0.0 b	0.00 b	0.0 c

^x Disease incidence based on percentage of plants displaying symptoms.

^y Disease severity rated on a scale of 0 to 5, where 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = moderate (30 to 50% of leaves) wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe (> 50% of leaves) wilt and necrosis, and 5 = death of plant.

^z Means within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

ease symptoms (severity index of 2.8) and, by 31 days after inoculation, were significantly stunted compared with plants inoculated with Vdt, Vdw, or Vdd, or the control (Table 2). The shoot weight of Vds-inoculated paprika plants also was significantly reduced compared with that of plants inoculated with the other three *V. dahliae* isolates or the controls. The root weight of Vds-inoculated paprika plants was significantly reduced compared with plants inoculated with Vdt and Vdw and the control (Table 2).

Tissue colonization. In the experiment in which three cultivars of paprika were inoculated with Vds and Vdp, plant tissues inoculated with Vds from all cultivars contained 10- to 35-fold more CFU in stems than in the leaves after 30 days. The fungal levels were 4.65, 2.34, and 3.57×10^4 CFU/g in stems, and 1.33, 1.25, and 3.44×10^3 CFU/g in leaves, in cultivars Shalhevet, Lehava, and Admoni, respectively. *V. dahliae* was not detected in plants inoculated with Vdp, or in the control (*data not shown*). From the root crown of Lehava plants inoculated with Vdt, Vdw, or Vdd, *V. dahliae* was recovered at significantly lower levels than from plants inoculated with Vds (47- to 4,706-fold; Table 2). The percentage of colonized stem segments in plants inoculated with Vdt, Vdw, or Vdd also was significantly lower than in Vds-inoculated plants. *V. dahliae* was not recovered from control plants (Table 2).

Pathogenicity of isolates in potato. Disease developed in potato plants inoculated with Vds and Vdp isolates. Within 10 days of inoculation, 95 and 80% of plants inoculated with Vds and Vdp, respectively, were showing symptoms of severe chlorosis and wilting. The number of stem segments producing growth of *V. dahliae* was significantly higher in potato plants inoculated with Vds (71.9%) than in those inoculated with Vdp (34%; Table 3).

Pathogenicity of isolates in eggplant. Both Vds and Vdp isolates of *V. dahliae* produced wilt symptoms in eggplant (Table 3), but symptom development was more rapid with Vds than with Vdp (*data not shown*). However, by 17 days after inoculation, all plants were showing symptoms, and the difference in disease severity between the two isolates was not statistically significant (Table 3). Symptoms were not observed in control plants. Colonization of the stem base of eggplants inoculated with Vds was significantly higher (6.4×10^3) than those inoculated with Vdp (1.52×10^3).

DISCUSSION

Although paprika has been grown in the Negev region for 15 years in fields known to be infested with *V. dahliae*, the recent outbreak of wilt and premature death is the first record of *V. dahliae* causing disease in paprika.

In this study, isolate Vds from diseased paprika was morphologically distinct from isolates Vdp, Vdt, Vdw, and Vdd. Vds grew more slowly in culture but produced microsclerotia earlier. Douira et al. (3) demonstrated similar differences between isolates of *V. dahliae* from pepper and from tomato.

Vds was highly pathogenic to potato, eggplant, and paprika, causing rapid disease development and severe symptoms, including stunting. Symptom development was correlated with high levels of tissue colonization. By contrast, Vdp was pathogenic to potato and eggplant, but disease symptoms did not develop and the fungus was unable to colonize paprika. Vdt, Vdw, and Vdd did not cause symptoms in paprika, although they colonized plant tissues.

These findings in regard to the response of paprika to *V. dahliae* isolates originating from tomato, watermelon, and *Dodonaea*, are in agreement with those of Ciccurese et al. (2) and Matta et al. (9); they reported that inoculation of pepper with *V. dahliae* isolates originating from chicory, broccoli, or tomato did not cause any disease symptoms, and concluded that pepper is immune to *V. dahliae*. On the other hand, Douira et al. (3) demonstrated that tomato isolates caused disease symptoms in pepper plants. Other research groups also demonstrated that *V. dahliae* isolates vary in pathogenicity to different hosts (1,4,12,13,17). Moreover, our results indicate that the absence of disease symptoms is not necessarily due to the inability of the fungus to penetrate or colonize the plant tissues. This conclusion is also supported by Fordyce and Green (6), who demonstrated that, although mint isolates of *V. dahliae* were not pathogenic to tomatoes, they were recoverable from the stems of inoculated tomato seedlings.

This outbreak of wilt in paprika due to *V. dahliae* is the first since the report of the isolation of *V. dahliae* from green pepper in Israel in 1982 (8). It is possible that variants of *V. dahliae* present in these fields have been selected over the last 15 years for pathogenicity to paprika (15), or that a new strain of *V. dahliae* may have been introduced. The high virulence of the paprika *V. dahliae* isolate to paprika, as well

as to other crops such as potato and eggplant, might influence the common crop rotations in Israel.

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