

# Two Sources of Resistance to Bacterial Speck of Tomato Caused by *Pseudomonas tomato*

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## ABSTRACT

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Cultivar susceptibility to *Pseudomonas tomato*, causal agent of bacterial speck of tomato, was tested under greenhouse and field conditions, with infested tomato seeds, infested soils, and spray-inoculated plants. Of 12 cultivars, Rehovot-13 had field resistance to the pathogen, VF 198 was most susceptible, and Hosen-Eilon was moderately resistant. In one greenhouse trial, cv. Marmande also showed high resistance.

Bacterial speck of tomato caused by *Pseudomonas tomato* (Okabe) Alstalt infects most of the tomato (*Lycopersicon esculentum*) cultivars in Israel (1,6). Economic damage particularly involves winter and spring crops covered with polyethylene.

Because of the expense of chemicals, resistant cultivars may be a better alternative for control. Pitblado and Kerr (7) noted resistance in cultivar ONT 7710. We report here greenhouse and field studies on the susceptibility of tomato cultivars to bacterial speck.

## MATERIALS AND METHODS

**Plants and growth conditions.** Tomato cultivars tested included the industrial cultivars VF 198, VF M-82-8-1, VF 134-1-2, and the fresh market cultivars Rehovot-13, Hosen-Eilon, Orit, and Naama 1684. They were obtained from the Hazera Co., Haifa, Israel. Seeds or seedlings were planted in medium heavy soil near Sandala, Yezreel Valley, in northern Israel.

The rows were covered with polyethylene tunnels, 50 cm high and 80 cm wide. Each test consisted of five randomized replicates. Each replicate consisted of 50 plants in a 10-m row. In the greenhouse, seeds of the above cultivars plus VF 145-513, VF 145B-7879, Mecheast 55, Homestead 24, and Marmande were planted in 1-L plastic pots (two plants per pot) containing 0.6 kg of uninfested sandy loam soil from Rehovot or soil from the site of the field experiments.

**Inoculation methods.** Either a local isolate of *P. tomato* or ATCC 10852 was used in all experiments (1,5). Inoculum

was prepared by incubating *P. tomato* in yeast peptone broth at 25 C (1). Seeds were infested by soaking samples in a *P. tomato* suspension containing  $1 \times 10^8$  colony forming units (CFU) per milliliter. The seeds were dried for 24 hr at 35 C (3).

Seedling leaves (three true leaves) were inoculated by spraying until runoff with a suspension of *P. tomato* ( $10^7$  CFU/ml) in either the field or greenhouse. A hand sprayer (Lehavot Habashan, Israel) was used in the field to spray soil surfaces with a suspension ( $5 \times 10^7$  CFU/ml) of the pathogen at the rate of 0.3 L/m<sup>2</sup>. The inoculum was incorporated into the soil by raking.

Soil in pots was infested with 100 ml of the same suspension mixed with the soil just before planting (3). Soil or leaves in the field were inoculated in the evenings, and the plots were sprinkle irrigated once every 3 days to promote infection. In the greenhouse, after inoculation, plants were incubated and periodically misted (5

sec/hr) to moisten the leaves to minimal runoff (1,3).

**Disease severity.** Disease severity was estimated either by the use of: 1) an index of 0 = no symptoms; 1 = 2-5 specks together or spread all over the leaf or fruit; 2 = 6-10 specks; 3 = more than 11 specks; or 2) by determining the percentage of diseased leaves or fruits. We examined six leaves per plant of a 30-plant sample (six plants per plot) during the growth season and 100 fruits (20 fruits per plot) at harvest. Any leaf or fruit showing infection was counted as positive for determining percentages.

## RESULTS AND DISCUSSION

Based on field tests, inoculated cv. Rehovot-13 had field resistance to bacterial speck of tomato and Hosen-Eilon was moderately resistant, whereas five other cultivars tested were highly susceptible (Table 1). Generally the incidence of fruit infection paralleled the incidence of leaf infection (Table 1), but damage to fruits was minimal.

To further demonstrate the field resistance of cv. Rehovot-13 to bacterial speck, plants were grown in the field by planting healthy seeds in soil infested by sprayed inoculum or by planting infested seeds in untreated (possibly infested) field soil. Disease index determinations and calculations of the percentage of diseased leaves clearly show that this cultivar has field resistance to bacterial speck of

**Table 1.** Response of seven tomato cultivars to artificial inoculation of an Israeli isolate of *Pseudomonas tomato*<sup>v</sup> under field conditions

Cultivar	Leaves			Percent infected after 20 days	Fruits	
	Disease index <sup>w</sup> days after inoculation				Disease index	Percent infected
	7	12	34			
Industrial						
VF 134-1-2	1.9 a <sup>x</sup>	2.5 a	1.5 b	69 <sup>y</sup>	0.82	60 <sup>z</sup>
VF M-82-1-8	1.5 a	2.5 a	1.5 b	60	0.88	56
VF 198	2.5 a	2.4 a	3.0 a	98	1.48	68
Fresh market						
Hosen-Eilon	0.8 b	1.0 c	1.5 b	33	0.40	28
Rehovot-13	0.4 c	0.3 d	0 c	10	0.18	18
Orit	2.0 a	1.5 b	0.8 b	79	0.93	55
Naama 1684	2.1 a	1.5 b	1.3 b	91	0.72	50

<sup>v</sup> A suspension of  $10^7$  CFU/ml sprayed over the leaves until runoff.

<sup>w</sup> Disease index: 0 = no symptoms; 1 = 2-5 specks together or spread all over the leaf or fruit; 2 = 6-10 specks; 3 = more than 11 specks. Index represents six leaves per plant.

<sup>x</sup> Numbers in columns followed by the same letter do not differ significantly at  $P = 0.05$ .

<sup>y</sup> Means of 30 plants (six plants per plot), approximately six leaves per plant.

<sup>z</sup> Means of 100 fruits (20 fruits per plot).

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**Table 2.** Disease index and percentage of infected Rehovot-13 leaves in the field after infestation of seeds or soil before planting

Treatment	Days after inoculation					
	50		98		125	
	Infection (%)	Disease index	Infection (%)	Disease index	Infection (%)	Disease index
Spray-infested soil, clean seeds <sup>a</sup>	12 <sup>b</sup>	0.12	10	0.2	4	0.04
Dip-infested seeds, untreated soil <sup>c</sup>	3	0.06	6	0.12	8	0.08
Control: clean seed, untreated soil	2	0.04	6	0.12	10	0.1

<sup>a</sup> Soils were sprayed with a suspension of *Pseudomonas tomato*,  $5 \times 10^7$  CFU/ml at a rate of 0.3 L/m<sup>2</sup>.

<sup>b</sup> Means of 30 plants (six plants per plot). Index represents six leaves per plant.

<sup>c</sup> Seeds were infested by soaking samples in *P. tomato* suspension containing  $1 \times 10^8$  CFU/ml. Seeds were dried for 24 hr at 35 C.

tomato (Table 2).

Clarification of whether resistance was due to conditions in the field or whether it was an inherent property of cv. Rehovot-13 was done as follows: Thirty plants (six true leaves) each of Rehovot-13 and VF 198 growing in infested soil from the field were transferred with their soil into pots in the greenhouse and incubated with periodic mist at  $25 \pm 2$  C and 12 hr daylight for 20 days. At these optimal conditions for disease development (1), no symptoms developed in Rehovot-13; VF 198 showed severe disease.

In an additional trial, plants were subjected to a continuous inoculum presence from all inoculum sources; eg, seeds, soil, foliage, and infected susceptible plants adjacent to the tested cultivar (1-3,6).

The experiment was done in 1-L pots (25 replicates); each pot contained two Rehovot-13 plants and two of the highly susceptible VF 198 plants. Plants originating from infested seeds and grown in infested soil were additionally

inoculated by spraying with the local isolate of *P. tomato* every 4 days. The plants were then incubated at  $25 \pm 2$  C under periodic mist and light conditions as described above. After 10 days, 80% of VF 198 plants showed a disease index of 3. Only 2% of the Rehovot-13 plants showed any infection (disease index < 1). Similar results were obtained in plants inoculated with *P. tomato* ATCC 10852.

In another greenhouse experiment (performed as previously described, in 10 replicates), Marmande, Homestead 24, VF 145B-7879, VF 145-513, and Mecheast 55 plants (three true leaves) were inoculated by spraying and incubated under periodic mist. Homestead 24 was susceptible to *P. tomato* (disease index = 3), whereas Marmande was resistant (disease index < 1). Seven days after inoculation, VF 145B-7879, VF 145-513, and Mecheast 55 were indexed as 1.8, 1.7, and 1.9, respectively; 20 days after inoculation, these cultivars showed 55, 65, and 72% of leaves infected, respectively.

Rehovot-13 was developed by N. Kedar

and co-workers in the Department of Vegetable and Field Crops, Faculty of Agriculture, Rehovot, and it is an F<sub>6</sub> generation of a single cross between Homestead 24 and Marmande. The cultivar is also resistant to *Fusarium oxysporum* f. *lycopersici*, race 1 (4).

The reason for spread of bacterial speck in Israel during the last 6-7 yr (5) may be related to changes in cultivar selection. In 1972-1973 Marmande was exchanged for Hosen-Eilon. Orit and Naama and the industrial cultivars VF 198, VF 134, etc, were introduced at about the same time from California. All of these recent selections except Hosen-Eilon are highly susceptible to *P. tomato*.

These findings should enable further work on genetical improvement of tomato cultivars for resistance to bacterial speck of tomato.

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