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Inhibition of seed germination and development of tomato plants in soil infested with *Pseudomonas tomato*

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SUMMARY

Infestation of sterilised or natural soil with *Pseudomonas tomato* at inoculum concentrations of 10^2 to 10^9 propagules/ml inhibited germination of seeds and caused damping-off of tomato cv. VF-198, susceptible to bacterial speck. Infestation with saprophytic *P. fluorescens* at an inoculum concentration of 10^9 propagules/ml did not cause any damage. Germination of seeds of tomato cv. Rehovot-13, resistant to *P. tomato*, was not affected in *P. tomato*-infested natural soil, but was inhibited when tested in *P. tomato*-infested, sterilised soil. Tomato plants which were symptomless from sowing to the flowering stage when growing in infested soil had 20–30% less foliage than plants growing in uninfested soil.

INTRODUCTION

Pseudomonas tomato (Okabe) Alstatt, the causal agent of bacterial speck in leaves and fruits of tomato (Goode & Sasser, 1980) survived for long periods on the surface of tomato seeds (Bashan, Okon & Henis, 1978; Okon *et al.*, 1979) and the pathogen developed from infested seedlings (Kim, 1979). Seeds were freed from the pathogen by heat treatment at 52 °C for 1 h (Devash, Okon & Henis, 1980).

Schneider & Grogan (1976) suggested that *P. tomato* behaved as a pathogen on tomato roots. However, there is no information available on the effect of the pathogen on seed germination and on the pre-emergence growth period.

The purpose of this study was to explore the pathogenicity of *P. tomato* towards tomato seeds and seedlings and to determine the effect of infested soil on plant development.

MATERIALS AND METHODS

Organisms. Pseudomonas tomato (Okabe) Alstatt (WT-1) isolated from diseased tomato plants and *Pseudomonas fluorescens* isolated from the natural phyllosphere microflora of tomato leaves were used in all experiments. Bacteria were grown in yeast-peptone broth at 30 °C for 24 h.

Tomato seeds (*Lycopersicon esculentum* Mill) cv. VF-198, highly susceptible to bacterial speck (Bashan *et al.*, 1978) and cv. Rehovot 13, resistant to the disease (Yunis, Bashan, Okon & Henis, 1980*a*) were obtained from Hazera Co., Haifa, Israel.

Seeds, soil and peat sterilisation. Seeds were surface-sterilised with 1% sodium hypochlorite under vacuum for 5 min. The vacuum was released abruptly to favour sterilisation. The seeds were then washed five times with sterile water in order to remove traces of hypochlorite. Soil and peat were autoclaved five times for 1 h, each time at 10^5 Nm^{-1}, with 24-h intervals at room temperature between each sterilisation (Devash *et al.*, 1980).

Germination methods. The seeds were germinated in sterilised or natural sandy loam soil from Rehovot in plastic pots, 500 g soil, 32 seeds/pot; in natural or sterilised rounded peat pellets (Jiffi-7) in fine nylon mesh (five seeds/pellet); or in Petri dishes containing 50 g of the same soil or cotton wool irrigated with soil extract (30 seeds/plate). Seeds were placed in position by using a seeding device (Henis, Ghaffar, Baker & Gillespie, 1978).

Infestation of soil, peat and Petri dishes. Pots of soil were infested with 20 ml of bacterial suspension containing 10^8 propagules/ml after sowing. Peat pellets were swollen in the same suspension before sowing. Soil or cotton wool in Petri dishes was infested with 10 ml bacterial suspension at concentrations of 10^2–10^9 propagules/ml after sowing. Controls were treated with water.

Estimation of disease severity. Development of cotyledons or roots from the seed was considered to be a positive indication of germination. Pre-emergence damping-off was evaluated by recording the proportion of seedlings emerging from the soil or peat. Seed germination was assessed after 5 days and seedling emergence after 14 days.

Growth conditions. Growth conditions of tomato plants and inoculum preparation were described by Bashan *et al.* (1978) and Okon, Bashan & Henis (1978). Seedlings from the emergence experiments were thinned down to one plant per pot and grown in the greenhouse until the appearance of the first flower.

All experiments were carried out in a growth chamber (25 ± 2 °C, 12 h light, 12 000 lux, 12 h dark) and each experiment in soil was conducted in 20 replicates and repeated three times whilst those in peat had four replicates and were also repeated three times.

Biomass determination. Leaves and stems were collected separately from five plants (2-months old) and dried at 80 °C for 7 days.

RESULTS

Effect of inoculum concentration on germination of tomato seeds in natural and sterilised soils. In a preliminary study it was found that high concentrations of *P. tomato* (10^8–10^9 propagules/ml) inhibited cotyledon and root emergence from 90% of the seeds of tomato cv. VF-198. Application of *P. fluorescens* (10^9 propagules/ml) did not cause any damage. When suspensions of *P. tomato* containing 10^2 to 10^8 propagules/ml were added to natural or sterilised sandy loam soil in Petri dishes, inhibition of seed germination increased as pathogen concentration increased. In sterilised soil seeds of both cvs VF-198 and Rehovot-13 were affected, whereas in natural soil germination was inhibited only in cv. VF-198 (Fig. 1*a*, *b*).

Effect on pre-emergence damping-off. Pots with sterilised or natural soil or peat pellets were irrigated with bacterial suspensions. In pre-sterilised soil and peat, pre-emergence damping-off developed in both cultivars (Table 1), whereas, in natural soil or peat, emergence of the resistant cv. Rehovot-13 was not affected. *P. tomato* was reisolated from each replicate at the end of each experiment from the soil.

Table 1. *Emergence of tomato seedlings in soil or peat infested with* Pseudomonas tomato

Tomato cultivar	Treatment	% emergence			
		Sterile soil	Natural soil	Sterile peat	Natural peat
VF-198	Infested	55·2a*	34·6a	60·0a	23·3a
	Uninfested	73·9c	75·0b	77·5b	67·5b
Rehovot-13	Infested	66·4b	66·6b	58·3a	68·3b
	Uninfested	79·2c	68·6b	80·8b	70·0b

* Different letters following numbers in each column differ significantly at $P = 0{\cdot}05$.

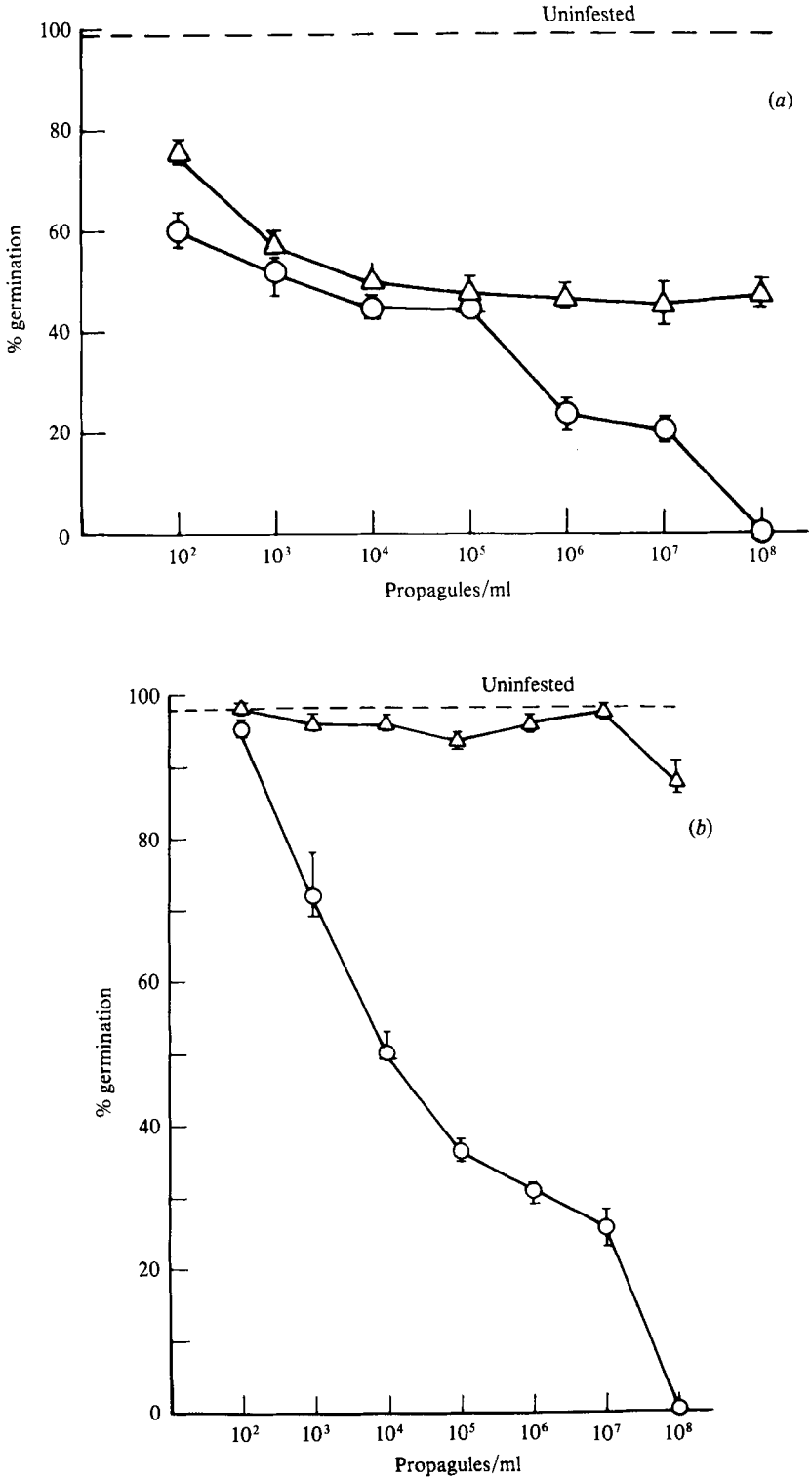

Fig. 1. Percentage of germination of tomato seeds in soil infested with different levels of *Pseudomonas tomato*. (*a*) sterilised soil. (*b*) natural soil, cv. VF-198 O——O, cv. Rehovot-13 △——△. Means from 3 experiments, 20 replicates each.

Table 2. *The effect of soil infestation with* Pseudomonas tomato *on dry weight of tomato plants*

Cultivar		Dry weight (g)					
		Sterile soil			Natural soil		
		Infested	Uninfested	% of control	Infested	Uninfested	% of control
VF-198	Leaves	5·05a*	6·30b	80·15	3·97a	5·63b	70·51
	Stems	2·87a	3·57b	80·4	2·18a	2·80b	77·85
Rehovot-13	Leaves	4·52a	4·72a	95·76	4·25a	4·42a	96·15
	Stems	2·85a	2·97a	95·95	2·55a	2·67a	95·50

* Numbers followed by different letters in each plant part and in each soil differ significantly at $P = 0{\cdot}05$.

Effect of P. tomato *on plant dry weight.* Seedlings from the emergence experiments were grown in the greenhouse for 2 months. Leaves remained symptomless throughout the growth period.

The dry weight of plants of cv. VF-198 growing in either sterilised or natural soil infested with *P. tomato* was reduced but cv. Rehovot-13 was not affected (Table 2). Thirty per cent reduction of plant height was observed under these conditions in cv. VF-198.

DISCUSSION

In this study it was demonstrated for the first time that soil infested with *Pseudomonas tomato*, the leaf pathogen of tomato plants, significantly affects tomato seeds at the pre-emergence stage. At the high inoculum levels used (10^8 propagules/ml) the pathogen completely prevented germination, and at lower inoculum levels (less than 10^4 propagules/ml) there was a significant decrease in percentage of seed germination.

Devash *et al.* (1980) demonstrated that high levels of *P. tomato* (10^6 propagules/plant) existed in nature, whereas populations of *P. tomato* as high as 10^6 propagules/g soil were detected in natural infested soils (Bosshard-Heer & Vogelsanger, 1977). When *P. tomato* alone was introduced at different inoculum levels to the soil or seeds, it was sufficient to incite leaf disease, thus, the pre-emergence damping-off demonstrated here was due to the pathogenicity of *P. tomato*, because very high concentrations of a saprophytic isolate of *Pseudomonas fluorescens* had no effect on germination. This phytopathological phenomenon was observed in both natural and sterilised soil as well as in peat pellets. Moreover, differences in susceptibility between cultivars were also observed. The seeds of the susceptible variety VF-198 were more affected than the seeds of the resistant cv. Rehovot-13, which was not affected in infested natural soil or in peat. This may indicate a different mechanism for foliage resistance and for seed resistance.

At present, pre-emergence damping-off of tomato seedlings is probably of relatively little economic importance, because tomato seeds are sown in excess. However, the decrease in germination and emergence, demonstrated in our experiments, may have greater economical significance in the future if more expensive seeds of F_1 cultivars are to be used.

Yunis, Bashan, Okon & Henis (1980*b*) found that when the foliage of field tomatoes was highly infected, there was a decrease in foliage weight, plant height and yield. Schneider & Grogan (1976) noted that when seedlings were soaked in bacterial suspensions and planted in sterile soil, plant height, stem and root weight decreased. In this work it was found that 200 seedlings which emerged in infested soil and remained symptomless for a further 8 wk had 20–30% less foliage. Thus, the yield obtained from these plants, would also be decreased.

Such an effect in symptomless plants is detectable only under greenhouse conditions such as described in this paper and rarely detectable in the field. Thus it would be difficult to distinguish

between decrease in crop yield due to other diseases and factors or by *P. tomato* at sublethal symptomless levels. The mechanisms involved in this phenomenon are currently under investigation.

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