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Department of Plants Genetics, The Weizmann Institute of Science, Rehovot 76 100, Israel

Inhibition of Seed Germination and Root Development Caused by *Xanthomonas campestris* pv. *vesicatoria* in Pepper and Tomato

YOAV BASHAN

Author's address: Dr. Y. BASHAN, Department of Plant Genetics, The Weizmann Institute of Science, Rehovot 76 100 (Israel).

With 4 figures

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Abstract

Inoculation of pepper seeds with the leaf pathogen *Xanthomonas campestris* pv. *vesicatoria* inhibited pepper germination. The inhibitory effect, which was stronger in non-sterilized light textured soils, decreased with time, and after 20 days or more, there was no difference between inoculated and non-inoculated seeds. Inhibitory substance(s) within the cytoplasmatic fraction of pathogen cells inhibited the germination of non-host tomato seeds. No relationship between pathogenicity to pepper leaves and inhibition of pepper seed germination was detected. The inhibitory substance(s) was found in two out of four *X. campestris* pv. *vesicatoria* strains. Heat-killed bacteria suppressed growth of pepper but not tomato seedlings. It is, therefore, suggested that the inhibition of seed germination and the decrease in root development are different modes of *X. campestris* pv. *vesicatoria* pathogenesis toward pepper plants.

Zusammenfassung

Hemmung der Samenkeimung und Wurzelentwicklung bei Paprika- und Tomatenpflanzen durch *Xanthomonas campestris* pv. *vesicatoria*

Die Inokulation von Paprikasamen mit dem Blattpathogen *Xanthomonas campestris* pv. *vesicatoria* hemmte die Paprikakeimung. Die hemmende Wirkung, die stärker in nicht sterilisiertem, leichtem Boden war, verringerte sich mit der Zeit und nach 20 oder mehr Tagen war kein Unterschied zwischen inokuliertem und nicht inokuliertem Samen festzustellen. Hemmende Substanz(en) in den

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zytoplasmatischen Fraktionen der Pathogenzellen hemmten die Keimung der Tomatensamen. Es wurde keine Beziehung zwischen der Pathogenität gegenüber Paprikablättern und der Hemmung der Paprikasamenkeimung gefunden. Hemmende Substanz(en) wurden in zwei von vier *X. campestris* pv. *vesicatoria*-Stämmen gefunden. Durch Wärme abgetötete Bakterien hielten das Wachstum von Paprika-, aber nicht von Tomatenkeimpflanzen zurück. Es wird daher angenommen, daß die Hemmung der Samenkeimung und die Verminderung der Wurzelentwicklung verschiedene Formen der Pathogenese von *X. campestris* pv. *vesicatoria* gegenüber Paprikapflanzen sind.

X. campestris pv. *vesicatoria* (Doidge) Dye, the causal agent of bacterial scab (leaf spot) in leaves and fruits of pepper and tomato, survives for long periods in their seeds (BASHAN *et al.* 1982a, b, BASHAN and ASSOULINE 1983, CROSSAN and MOREHART 1964, KRUPKA and CROSSAN 1956, LEWIS and BROWN 1961). Infected seeds produce diseased seedlings, under appropriate conditions, and diseased seedlings, with or without symptoms can develop into diseased plants (BASHAN *et al.* 1984, 1985, DIAB *et al.* 1982, KRUPKA and CROSSAN 1956, LEBEN 1962, LEWIS and BROWN 1961, PETERSON 1963, SHARON *et al.* 1982). Though the pathogen can be eliminated from the seeds by chemical treatment (DEMPSEY and WALKER 1973), the Israeli seed industry has not adopted the procedure. *X. campestris* pv. *vesicatoria* is not known as a root or seed pathogen and, to the best of my knowledge, there is no information available on its effect on seed germination and root development. Inhibition of seed germination and decrease in plant growth, as a result of seed infection, was demonstrated for *Pseudomonas syringae* pv. *tomato*, the causal agent of bacterial speck of tomato (BASHAN and OKON 1981). Association between *P. syringae* pv. *lachrymans*, the causal agent of angular leaf spot of cucumber, and other bacteria with cucumber roots was suggested by LEBEN (1983). However, the effect(s) of the bacteria existing in the root system on plant growth was not determined.

The purpose of this study was to study the pathogenicity of *X. campestris* pv. *vesicatoria* towards pepper seeds and seedlings.

Materials and Methods

Organisms and growth conditions

X. campestris pv. *vesicatoria* (R-1, R-2, R-3 and R-4), pathogenic to pepper but not to tomato plants, were isolated from diseased pepper plants from the Jordan Valley and used in all experiments (DIAB *et al.* 1982). Heat-killed bacteria were produced by heating twice-washed bacterial culture at 100 °C for 60 min. Pepper seeds (*Capsicum annuum*) cv. "Ma'or", which are highly susceptible to bacterial scab (BASHAN *et al.* 1984) and tomato seeds (*Lycopersicon esculentum*) cv. "VF-198" and cv. "Rehovot-13", which are highly susceptible and resistant, respectively, to bacterial speck of tomato (YUNIS *et al.* 1980), were obtained from Hazera Co. Haifa, Israel.

Seeds (five seeds or seedlings in each pot or Petri dish) were sown by the following methods: a) in Petri plates containing filter paper soaked in Hoagland's nutrient solution; b) in Petri dishes containing sterilized or natural soils maintained near field capacity throughout the experiment; c) in pots (10 × 10 × 10 cm) containing 500 g volcanic dust (0.1—8 mm particle size) irrigated with Hoagland's nutrient solution.

Germination experiments were carried out in an incubator at 30 ± 2 °C or 22 ± 2 °C. Plants were grown in an air-conditioned glasshouse. Growth conditions, for both bacteria and plants, were described by DIAB *et al.* 1982.

The effect on germination was tested in various soil types, all taken from areas near pepper fields (details are given in Table 2). Rendzina-of-valleys soil untreated for several years, with any synthetic chemicals was considered "Organic" soil. It was fertilized with organic compost only. Weeds, insects and diseases were controlled by natural enemies or other biological means (LEVY 1979).

Seeds, soil and volcanic dust disinfection

Seeds were surface-disinfected with 1 % sodium-hypochlorite under vacuum for 2 min. The vacuum was released abruptly to favor disinfection. The seeds were then washed five times with sterile tap water, in order to remove traces of hypochlorites, and then dried. Soil and volcanic dust were autoclaved three times, each for 1 h, at 1.2 Atm. with 24 h intervals at room temperature, between each disinfection (BASHAN and OKON 1981). Alternatively, soil was either mixed with 10 % benzen (5:1, v/v) which was later evaporated in a flew hood or gamma irradiated (2.5 mega rad).

Infestation of soils and volcanic dust

Pots were infested with 20 ml of bacterial suspension containing 10^7 colony-forming units (CFU)/ml after sowing. Soil in Petri dishes was infested with 10 ml bacterial suspension at a concentration of 10^2—10^9 CFU/ml after sowing. Controls were treated with sterile tap water. Inoculation of plant leaves was carried out according to DIAB *et al.* (1982).

Germination index and percentage of germination measurements

The germination index was measured using the following scale: 0 — no germination; 1 — beginning of visible germination; 2 — small seedling (< 5 mm); 3 — seedling longer than 5 mm. Germination percentage was recorded at germination index of one or more.

Fractionation of bacterial cells

X. campestris pv. *vesicatoria* cells (10^{10} CFU/ml) grown in yeast-peptone liquid broth (DIAB *et al.* 1982) were harvested by centrifugation, washed five times in a sterile 0.03 M phosphate buffer, pH 7.0, adjusted to the initial bacterial concentration in the same buffer and sonicated for 15 min at 140 V in an ice bath using an ultrasonic disintegrator (Sonifier B-12, Branson Sonic Power, Conn.). The sonicate was subjected to four successive centrifugations at 30,000 xg for 30 min each. After each centrifugation the pellet was collected and the supernatant was subjected to another centrifugation. The two fractions (pellet and supernatant) were lyophilized to dryness and kept at —20 °C until needed, at which time they were redissolved to 0.001 % of their original concentration before application to germinating seeds.

Biomass determination and root-surface area measurements

Leaves and roots were collected, dried separately at 80 °C for 72 h in an air forced oven and weighed immediately. Root-surface area measurements was carried out by a gravimetric method using $Ca(NO_3)_2$ (CARLEY and WATSON 1966).

Experiment design and statistical analysis

All experiments were repeated two to three times, each in 10 replicates using 10 pots or 10 Petri dishes as a replicate. Results are from a representative experiment. Significance is given by $P \leq 0.05$ using Duncan's Multiple Range Test.

Results

Effect of inoculum concentration on pepper seed germination

The inhibitory effect of *X. campestris* pv. *vesicatoria* R-3 on germinating pepper seeds was measured in Petri dishes containing soil. Though a relatively

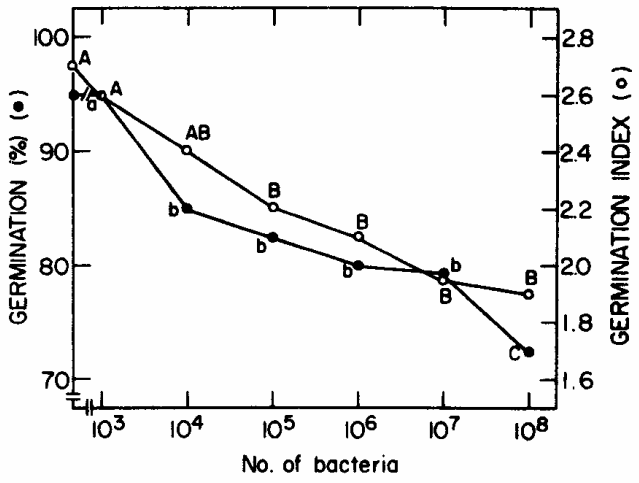

Fig. 1. Effect of different concentrations of *X. campestris* pv. *vesicatoria* inoculum on percentage of germination (●) and germination index (○) of pepper seeds. Points on each graph (separately) followed by different letters differ significantly at $P \leq 0.05$.

low bacterial concentration (10^3 CFU/ml) had no apparent effect on germination, the percentage of germination inhibition increased with the increase in bacterial concentration, while the germination index of the seeds, indicating their germination potential, decreased (Fig. 1). However, 20 or more days after inoculation, no difference in germination percentage or germination index could be detected between inoculated and non-inoculated seeds.

Effect of *X. campestris* pv. *vesicatoria* inoculation on the germination rate of pepper and tomato seeds

Pepper and tomato seeds were inoculated with washed live *X. campestris* pv. *vesicatoria* (10^7 CFU/ml) or heat-killed bacteria in Petri dishes containing filter paper. Germination index and percentage were inspected daily. Control seeds were treated with sterile tap water.

X. campestris pv. *vesicatoria* influenced the germination process. Heat-killed *X. campestris* pv. *vesicatoria* had the most marked effect on germination, especially in tomato seeds (Fig. 2 C and D), whereas live bacteria had no effect on tomato seed germination. Figures 2 A and B show that pepper seeds overcame the temporary inhibitory effect caused by either live or dead bacteria, but tomato seeds did not, and were inhibited by *X. campestris* pv. *vesicatoria* throughout the experiment.

Inhibition of pepper seed germination by *X. campestris* pv. *vesicatoria* in sterile and non-sterile soil and in various soil types

Brown-red degrading soils were subjected to three sterilization methods — tyndelization, benzene evaporation and Gamma irradiation. Inhibition of germination was detected in all *X. campestris* pv. *vesicatoria*-inoculated treatments as compared to uninoculated soils. However, the most marked effect was obtained in non-sterilized soil (Table 1).

When comparing different soil types, the inhibitory effect appeared more severely in light textured soils (inhibition of 72—78 %), whereas in medium and

heavy soils there was only 20—35 % inhibition. In soil treated by "biological" agriculture, no inhibitory effect was observed (Table 2).

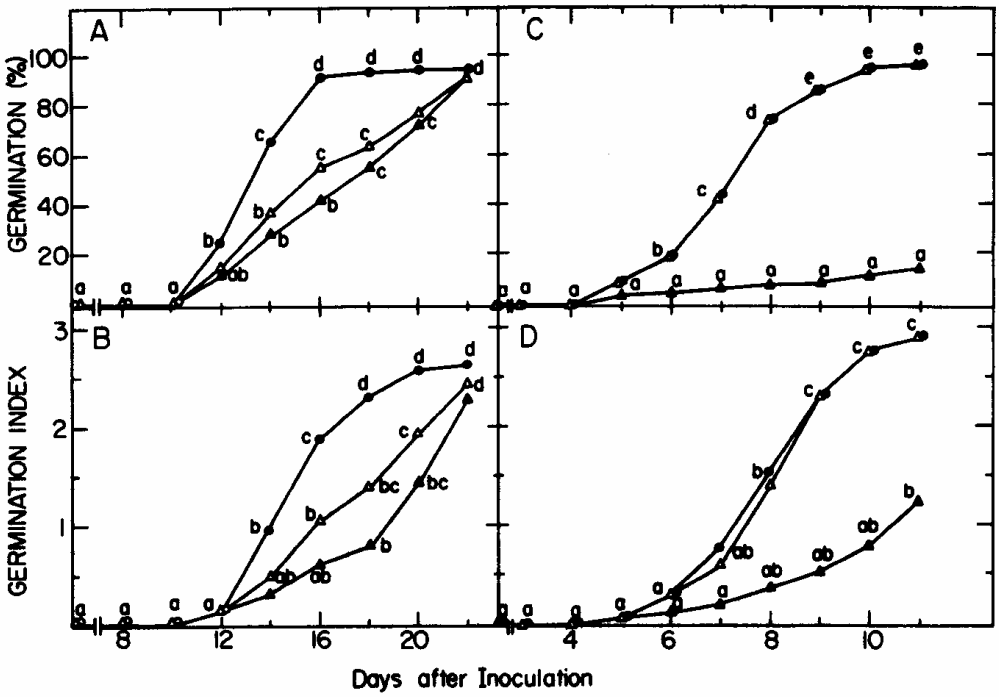

Fig. 2. Effect of inoculating pepper and tomato seeds by *X. campestris* pv. *vesicatoria* with time. Percentage of germination, A-pepper, C-tomato. Germination index, B-pepper, D-tomato. (Δ) — inoculation with live bacteria; (▲) inoculation with heat-killed bacteria; (●) — sterile water. Points in each sub-figure (separately) followed by different letters differ significantly at $P \leq 0.05$.

Table 1

Effect of *X. campestris* pv. *vesicatoria* (R-3) on germination of pepper seeds inoculated after soil[a] sterilization

Soil sterilization	Inoculated seed		Uninoculated control	
	Germination percentage	Germination index[e, f]	Germination percentage	Germination index[e, f]
Tyndelization[b]	56.4 b[g]	1.36 b	83.6 c	2.81 d
Benzen[c]	59.1 b	1.44 b	80.4 c	2.72 d
Gamma irradiation[d]	66.4 b	1.27 b	91.3 c	2.88 d
Untreated	26.2 a	0.52 a	62.6 b	2.0 c

[a] Brown-red degrading soil.
[b] 1 h—1 atm—121 °C three times with 24 hr intervals at 30 °C between treatments.
[c] Single treatment.
[d] 2.5 mega-rad.
[e] Germination index; 0-no germination; 3-normal germination.
[f] 16 days after sowing.
[g] Numbers representing germination percentage or index (separately) followed by different letters differ significantly at $P \leq 0.05$.

Table 2
Effect of *X. campestris* pv. *vesicatoria* (R-3) on germination of pepper seeds in various untreated soil types after 16 days

Soil type[a]	Location	Inoculated seeds		Uninoculated control	
		Germination percentage	Germination index[b]	Germination percentage	Germination index
Loess raw soil	Nir-Am, Northwestern Negev	72.4 bc[c]	1.55 b	88.7 c	2.66 c
Alluvial soil	Negba, Northern Negev	68.6 b	1.49 b	81.1 c	2.57 c
Loessial sandy soil	Gevulot, Central Western Negev	24.2 a	0.51 a	80.4 c	2.63 c
Brown-red sandy soil alternating with "Kurkar"	Habonim, Coastal Plain	23.6 a	0.47 a	82.9 c	2.96 c
Terra rossa soil	Daburia, Tabor Mountain	70.1 bc	1.61 b	90.2 c	2.44 c
Brown-red degrading soil	Ra'anana, Sharon Region	27.6 a	0.66 a	78.2 bc	2.73 c
Brown alluvial soil of basaltic origin	Yavne'el Valley	73.8 bc	1.51 b	86.6 c	2.47 c
Rendzina valleys soils[d]	Sedeh-Eliahu, Bet-She'an Valley	80.6 c	2.43 c	83.9 c	2.66 c
Brown basaltic soil	Golan Heights	28.1 a	0.52 a	85.8 c	2.82 c
Volcanic dust	Fares Volcano, Golan Heights	22.7 a	0.48 a	86.1 c	2.88 c
Desert alluvial soil	Tomer, Jordan Valley	65.4 b	1.46 b	81.6 c	2.61 c

[a] According to RAVIKOVITCH (1981).
[b] Germination index; 0-no germination; 3-normal germination.
[c] Numbers followed by different letters in each parameter (separately) differ significantly at $P \leq 0.05$.
[d] "Biological" soil (LEVY 1979).

Effect of *X. campestris* pv. *vesicatoria* cell-fractions on tomato and pepper seed germination

Cell-wall and cytoplasmatic fractions were applied to germinating tomato and pepper seeds (3 ml solution per plate) maintained on filter paper in Petri dishes.

The cell-wall fraction had no apparent inhibitory effect on seeds of both types. However, the cytoplasmatic fraction inhibited seed germination. Tomato seeds were almost totally inhibited and did not recover with time (Fig. 3 A). Pepper seed germination, though almost completely inhibited for the first 14 days after treatment, overcame this inhibition and 22 days after inoculation there was

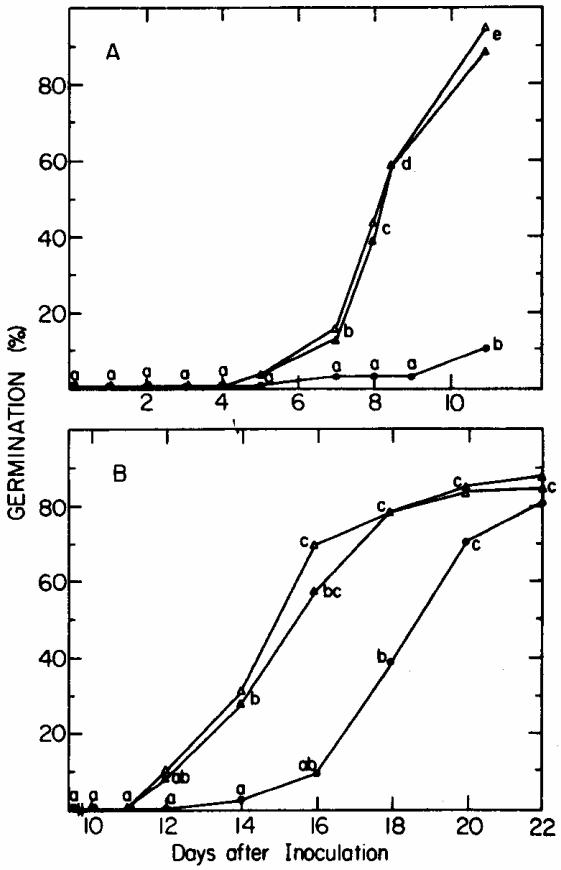

Fig. 3. Effect of cell-wall (▲) and cytoplasmatic (●) fractions of *X. campestris* pv. *vesicatoria* on germination of tomato (A) and pepper (B) seeds. (Δ) sterile water. Points in each sub-figure (separately) followed by different letters differ significantly at $P \leq 0.05$.

no difference in germination percentage between treated and untreated seeds (Fig. 3 B).

Relationship between pathogenicity to leaves and inhibition of seed germination

The four *X. campestris* pv. *vesicatoria* isolates tested for pathogenicity to leaves and to germinating seeds, using 10^7 CFU/ml at the log phase of growth, produced the following disease indexes: R-1: 1.46 ± 0.12; R-2: 2.86 ± 0.08; R-3: 2.78 ± 0.14; R-4: 1.76 ± 0.17. Only *X. campestris* pv. *vesicatoria* R-3 had a marked inhibitory effect on seed germination. Isolates R-1 and R-2 had no effect whereas isolate R-4 had only a light inhibitory effect (12 ± 3 % of the relative effect of R-3 in three experiments).

Effect of live and heat-killed *X. campestris* pv. *vesicatoria* on dry weight of leaves and roots and on root-surface area of tomato and pepper seedlings

Two *X. campestris* pv. *vesicatoria* isolates (R-2 and R-3), which did not differ in their pathogenicity towards pepper leaves, were tested for their effect on the growth of tomato and pepper seedlings. The addition of the incompatible *X. campestris* pv. *vesicatoria* isolates to tomato seedlings enhanced their growth

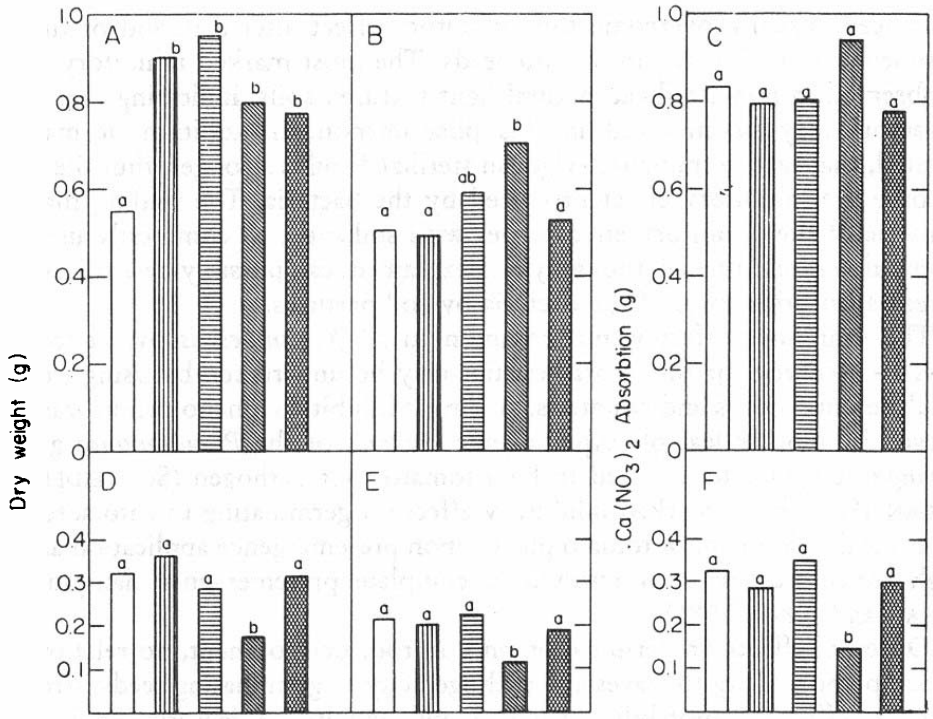

Fig. 4. Effects of *X. campestris* pv. *vesicatoria* R-2, R-3 and heat-killed bacteria on dry weight of tomato leaves (A) and roots (B), tomato root-surface area (C), dry weight of pepper leaves (D) and roots (E) and pepper root-surface area (F). Each sub-figure contains (from left to right): non-inoculated control □, inoculation with *X. campestris* pv. *vesicatoria* R-3 ▥, inoculation with *X. campestris* pv. *vesicatoria* R-2 ▤, inoculation with heat-killed *X. campestris* pv. *vesicatoria* R-3 ■, inoculation with heat-killed *X. campestris* pv. *vesicatoria* R-2 ■. Histograms followed by different letters in each sub-figure (separately) differ significantly at $P \leq 0.05$.

without visilbe attack (Fig. 4 A and B). No significant difference ($P \leq 0.05$) was found between surface area of roots from inoculated and uninoculated soils (Fig. 4 C).

A different pattern of phenomena was observed by inoculating the root system of the compatible pepper plants. Heat-killed isolate R-3 significantly decreased the dry weight of leaves and roots and root-surface area. In contrast, heat-killed isolate R-2 (similar in pathogenic ability) had no marked effect on the three tested parameters. Applying live *X. campestris* pv. *vesicatoria* bacteria to the soil had no significant effect on the growth parameters of growing seedlings (Fig. 4 D, E, F).

Discussion

X. campestris pv. *vesicatoria*, the leaf pathogen of tomato and pepper plants is known to be seedborne.

This study demonstrates that a pepper-specific isolate can unspecifically inhibit germination of either incompatible tomato or compatible pepper seeds.

Pepper seeds usually overcome this inhibitory effect after a period of time, a phenomenon not observed in tomato seeds. The most marked inhibitory effect was observed in non-sterilized natural light textured soils, indicating that other soil factors may be involved in this phenomenon. In addition, it may be suggested, that seed germination-vigor in sterilized soil is stronger, thus the seeds overcome the inhibitory effect produced by the bacteria. The finding that the magnitude of the inhibitory effect varies with soil type, all commonly used for pepper cultivation in Israel, though yet unexplained, can possibly be attributed to different absorption rates of the bacteria by soil particles.

The inhibitory effect is not common to all *X. campestris* pv. *vesicatoria* strains — a survey for this characteristics may be undertaken by using isolates from different sources and countries. A similar inhibitory phenomenon was first observed in another leaf phytopathogenic bacteria of the *Pseudomonas* group. *P. syringae* pv. *tomato*, claimed to be a tomato root pathogen (SCHNEIDER and GROGAN 1976), has a marked inhibitory effect on germinating tomato seedlings and on the development of tomato plants upon pre-emergence application and, at a high concentration, even resulted in complete pre-emergence damping off (BASHAN and OKON 1981).

Different effects on germination and on root development, no relationship between pathogenicity to leaves and pathogenicity to germinating seeds, stronger inhibitory effect of heat-killed bacteria and activity of factor(s) in bacteria cytoplasm, all indicate that different mechanisms of pathogenicity may be involved in inducing the inhibitory phenomenon compared to the pathogenicity of *X. campestris* pv. *vesicatoria* towards pepper leaves.

At present, inhibition of seed germination is probably of relatively little economic importance because tomato and pepper seeds are not very expensive and are sown in excess. However, the inhibition of germination may have a greater economic significance in the future with the increased use of the more expensive F_1 cultivars seeds. The inhibitory effect, resulting in smaller seedlings at marketing time, may also cause damage to vegetable nurseries growing seedlings in "speedling" trays under fully automatic growth conditions, by eliminating these seedlings from selling. Additionally, yields destinated for export at a precise date may end up, at least partially, in the local fresh market as a result of this inhibition. The exact nature of the factors involved in the inhibitory phenomenon should be further investigated.

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