# Long-Term Survival of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in Tomato and Pepper Seeds

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## ABSTRACT

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*Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria*, the causal agents of bacterial speck of tomato and bacterial scab of pepper, survived on dried seeds for 20 and 10 yr, respectively. Seedlings from old infested seeds either developed visible disease symptoms or no symptoms. Symptomless plants grown under high relative humidity contained massive populations of the pathogen.

Long-term survival of phytopathogenic bacteria in seeds of vegetables has a significant economic importance for both seed production companies and growers. Dissemination of infested seeds in plots with no disease history has often resulted in an unnecessary damage (7,13).

*Pseudomonas syringae* pv. *tomato* (Okabe 1933) Young, Dye, and Wilkie 1978, the causal agent of bacterial speck of tomato, persists on host seeds as cell aggregates inside holes and cavities and can produce diseased seedlings if seeds are germinated in mist chambers (4). Furthermore, when introduced to tomato fields in infested seeds, *P. syringae* pv. *tomato* was established in the soil and infested the growing plants (1).

Pepper seeds infested with *Xanthomonas campestris* pv. *vesicatoria* (Doidge 1920) Dye 1978b are known as major sources of inocula for bacterial scab of pepper (3,5,6,17). The common hypothesis for the phenomenon of long-term survival of pathogenic bacteria suggests that the pathogen persists by passing into a hypobiotic state, a state of substantially reduced metabolism (10). However, this hypothesis has not been verified.

The purpose of this study was to further examine the relation between the hypobiosis theory and the survival of these phytopathogenic bacteria on seeds.

## MATERIALS AND METHODS

**Organisms and growth conditions.** We tested commercial summer crops of pepper and tomato seeds harvested at various seasons. Tomato cultivars and years harvested were: Marmande, 1962 and 1972; VF-198, 1975, 1976, and 1977; VF-145-513, 1976; VF-145B-7879, 1976; M-82, 1977; Rehovot 13, 1976; VF-317, 1976; Mecheast 55, 1976; VF-134-1, 1976; VF-134-1-2, 1976. Pepper cultivars harvested were: Ma'or, 1977, 1978, and 1979; and Kalenkov, 1972. Seeds were sown in plastic pots (100 seeds per pot, 10 replicates) containing 600 g of a mixture of tuff and peat (1:1, v/v) and covered with a 1-cm layer of sterilized vermiculite. The pots were transferred to a mist chamber (5 sec of misting every 30 min), where the tomatoes remained at $25 \pm 3$ C for 10 days in the case of tomato or 20 days in the case of pepper (1).

**Evaluation of disease development.** The percentage of diseased seedlings from seeds germinated in the mist chamber was recorded. Eight hundred seeds were placed in 10 ml of 0.06 M potassium phosphate buffer (10 replicates) for 5 hr and shaken vigorously. Then, 2-ml aliquots were placed on surface-disinfested, detached tomato or pepper leaves; these were incubated to check for the presence of the pathogens by the recently developed enrichment procedure (8,16). This method used the host's leaves as an enrichment medium and is based on selective multiplication of the compatible pathogen within the host tissue, whereas nonpathogenic bacteria or saprophytic bacteria multiply only on the leaf surface.

Six days later, leaves were homogenized in a sterile Omni-Mixer (Sorvall), and the homogenate was serially diluted and spread on nutrient agar (Difco) in the case of *X. campestris* pv. *vesicatoria* or on King's medium B (9) in the case of *P. syringae* pv. *tomato*. Plates were incubated at 30 C for 48 hr. Control plates for the above method were prepared from homogenates of leaves treated with sterile water, surface-disinfested seeds (pretreated with 3% NaOCl for 5 min and washed five times with sterile water), or from uninoculated leaves. Suspected colonies were tested for pathogenicity (1). Procedures used for inoculum preparation and mist chamber conditions were as described elsewhere (1,4,12).

**Germination test.** One hundred seeds (in 10 replicates) were treated with Thiram (TMTD) 50% powder (2 mg/g seeds) to prevent contamination with fungi and placed on Whatman No. 1 filter paper disks (9-cm-diameter) that had been soaked with 5 ml of distilled water. The seeds were incubated in a growth chamber (16 hr light, 5,000 lux, 25 C). Germination was recorded at the end of 10 days for tomato seeds and 20 days for pepper seeds.

**Bacterial counts from leaves and seeds.** The presence of pathogenic bacteria in seeds and leaves of tomato and pepper was examined. Three grams of seeds or 2 g of leaves (three replicates from each sample) were homogenized for 3 min in sterile phosphate buffer in an Omni-Mixer immersed in an ice bath. Bacterial populations were determined by the dilution plate count method (18).

**Seed infestation and storage.** Tomato seeds were infested by soaking 10-g samples in 10 ml of solution containing $10^9$ *P. syringae* pv. *tomato* colony-forming units (cfu) per milliliter under water pump vacuum. The vacuum was abruptly broken to obtain penetration of bacteria into seed cavities. Seeds were air-dried for 24 hr in a laminar-flow hood at 35 C. Other samples of naturally infested commercial tomato and pepper seeds were obtained from diseased fields; infestation was indicated because their emerging

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TABLE 1. Long-term survival of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper seeds

| Cultivar and pathogen | Storage time (years) | Diseased seedlings[w] (%) | Pathogen after enrichment[x] (cfu/g leaf) | Pathoge-nicity test[y] | Germi-nation[z] (%) |
|---|---|---|---|---|---|
| Tomato—*P. syringae* pv. *tomato* | | | | | |
| VF-317 | 6 | 2 a[t] | $2.6 \times 10^6$ c | + | 85 |
| VF-198 | 5 | 13 b | $8.1 \times 10^8$ d | + | 71.6 |
| VF-198 | 7 | 0 a | 0 a | − | 92 |
| Marmande | 10 | 4 a | $6.0 \times 10^5$ b | + | 71 |
| Marmande | 20 | 1 a | $4.8 \times 10^5$ b | + | 86.6 |
| M-82 | 3 | 0 a | 0 a | − | 88 |
| VF-134-1-2 | 6 | 0 a | 0 a | − | 86 |
| Rehovot-13 | 6 | 0 a | 0 a | − | 89.1 |
| VF-198[u] | 6 | 42 c | $7.4 \times 10^8$ d | + | 65.1 |
| VF-134-1[u] | 6 | 60 c | $2.9 \times 10^8$ d | + | 84.1 |
| VF-145-513[u] | 6 | 7 b | $3.1 \times 10^6$ c | + | 83 |
| VF-145B-7879[u] | 6 | 5 ab | $4.7 \times 10^6$ c | + | 87 |
| Mecheast 55[u] | 6 | 4 a | $1.1 \times 10^5$ b | + | 90.2 |
| Pepper — *X. campestris* pv. *vesicatoria* | | | | | |
| Ma'or[v] | 2 | 5 b | $2.6 \times 10^5$ b | + | 92 |
| Ma'or | 3 | 0 a | 0 a | − | 88 |
| Ma'or | 4 | 3 b | $1.8 \times 10^5$ b | + | 73 |
| Kalenkov | 10 | 0.66 a | $8.2 \times 10^4$ b | + | 37.3 |

[t] Numbers followed by different letters in the same column (for every plant-pathogen interaction) differ significantly at $P = 0.05$.
[u] Artificial infestation.
[v] 16 samples, each from a different field.
[w] Triplicate samples each of 1,000 seedlings were examined for symptoms.
[x] Triplicates, 2 g of leaves (fresh weight) for each replicate.
[y] Three plants (with four true leaves) were sprayed with the compatible pathogen ($10^8$ cfu/ml) until runoff and incubated in a mist chamber for 8 days.
[z] Five replicates, 100 seeds for each replicate.

seedlings showed bacterial speck or scab symptoms in commercial fields during the growing season following seed production. Dried seeds were vacuum packed and stored on the shelf for several years in hermetically sealed tin boxes (200 g of seed per box) at 20 ± 10 C.

## RESULTS AND DISCUSSION

Infested seeds are important sources of pathogenic bacterial infection of plants. Seeds play a double role in the spread of bacterial diseases. They provided a site for overwintering of bacteria and for infection of seedlings, and they may transmit bacteria over long distances (7,14). The pathogens that cause bacterial speck of tomato and bacterial scab of pepper are seedborne (3,4,17); therefore, eliminating them is rather difficult (4,16). It is known that *P. syringae* pv. *tomato* can survive on dried tomato seeds for at least 6 mo (4) and that *X. campestris* pv. *vesicatoria* can survive on tomato seeds for up to 16.5 mo (5,6).

The hypobiosis theory of Leben (10,11) suggests that bacteria survive in a state of reduced metabolism. The theory has not been satisfactorily substantiated, possibly for technical reasons involving the difficulty in planning experiments that continue for several years. However, some reports indicate that *Corynebacterium flaccumfaciens* pv. *flaccumfaciens* and *X. campestris* pv. *phaseoli* can survive on bean seeds for between 3 and 24 yr (2,14,15,20). When attempts to detect pathogenic bacteria by the dilution count plate method (in which seed homogenates are plated on solid agar medium) failed, the leaf enrichment method (8,16) was used. Thirty-two seed lots were checked after several years of storage. Some of the naturally infested seed lots were found to be contaminated with the compatible pathogens. These pathogens initiated disease symptoms either in the emerging seedlings inoculated and incubated in mist chamber conditions, or on detached leaves. All the 6-yr-old, artificially infested tomato seed lots contained the pathogenic *P. syringae* pv. *tomato*. Furthermore, seed lots that generated higher percentages of diseased seedlings, generally showed a higher rate of multiplication of the pathogen in detached leaves (Table 1). The evidence provided here showed that in Israel the main bacterial disease agents of tomato and pepper can survive for long periods of time and gave support to the hypobiosis hypothesis. The seeds had been infested once and subsequently had been maintained for many years under conditions unfavorable for bacterial development.

*P. syringae* pv. *tomato* was first detected in Israel in 1971 (19). Its presence in 20-yr-old seeds suggested that it is a local pathogen. The increase in bacterial speck severity during the last 10 yr may have resulted from a change from resistant cultivars to susceptible cultivars.

Results of this study suggest that bacteria in "dried" form can survive for long periods of time. Thus, old seed stocks maintained for breeding programs and cultivar collections may be sources of dormant "latent" infestations that can be "awakened" and initiate disease in susceptible plants. They should be tested for presence of bacterial pathogens before being used.

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