Comparative Growth of Xanthomonas phaseoli and Xanthomonas vesicatoria and Development of Symptoms in Bean and Tomato Leaves

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This work was supported in part by a fellowship from the Rockefeller Foundation to the senior author.

Accepted for publication 18 October 1971.

ABSTRACT

The growth patterns of *Xanthomonas phaseoli* and *Xanthomonas vesicatoria* in their natural hosts, bean and tomato, respectively, were similar. After inoculation by the injection-infiltration method, populations of the bacteria initially increased rapidly in the leaves, then gradually decreased. The maximal numbers of the pathogens in the leaves was attained more rapidly after inoculation with 10^8 cells/ml than with 10^6 cells/ml, and tissue necroses appeared when the populations reached the maxima.

The population trends of *X. phaseoli* and *X. vesicatoria* in their nonhosts, tomato and bean, respectively, were different. The population of *X. phaseoli* decreased rapidly in tomato leaves, whereas the population of *X. vesicatoria* increased in bean leaves. Both bacteria induced visible necrosis in leaves of their nonhost plants at an inoculum concentration of 10^8 cells/ml, but not at 10^6 cells/ml. *Xanthomonas phaseoli* caused a rapid development of necrosis in tomato leaves, whereas *X. vesicatoria* caused a slow development of necrosis in bean leaves.

Phytopathology 62:329-332.

Additional key words: Phaseolus vulgaris, Lycopersicon esculentum.

Several investigators have shown that plant-pathogenic bacteria multiply, produce high populations, and cause typical symptoms when inoculated into their natural host plants, but fail to continue to multiply and cause only atypical necroses when inoculated into nonhost or incompatible plants (2, 5). The rapid termination of growth of a bacterial pathogen in a nonhost after an initial population increase is considered characteristic of a hypersensitive reaction. Hypersensitive reaction of plants to infection by bacterial plant pathogens occurs commonly in the incompatible host-pathogen relationships. This hypersensitive response is considered to be a defense mechanism which may play a role in limiting the further development of the pathogen in incompatible plants (5). These observations suggest that factors influencing multiplication of pathogens in vivo may be associated with pathogenesis.

This investigation compared the growth of *Xanthomonas phaseoli*, causal agent of common blight of bean, and *Xanthomonas vesicatoria*, causal agent of bacterial leaf spot of tomato, in compatible and incompatible host plants, and the development of symptoms by the hosts.

MATERIALS AND METHODS.—Virulent isolates

of *X. phaseoli* (XP-24) and *X. vesicatoria* (XV-21) were used and maintained on potato-dextrose agar (pH 7.0) which contained 1% dextrose. Bacterial cultures were grown in flasks containing Difco nutrient broth plus 0.5% glucose and 0.5% yeast extract at 28 C for 24 hr in a water bath shaker (100 strokes/min). The cultures were centrifuged at about 5,000 *g* for 15 min, and the pellets washed twice and suspended in sterile distilled water. The inoculum was adjusted to ca. 10^8 viable cells/ml by optical density at 620 nm with a Spectronic 20 colorimeter; suspensions containing 10^6 cells/ml were prepared by diluting the adjusted suspensions.

Bean (*Phaseolus vulgaris* L. 'Red Kidney') and tomato (*Lycopersicon esculentum* Mill. 'Valiant') were used in all tests. The seeds were germinated in steam-treated vermiculite, and ca. 9-day-old bean and 21-day-old tomato seedlings were selected for uniformity and were transplanted to 4-inch pots (2 bean seedlings or 1 tomato seedling/pot) containing steam-treated soil. The bean plants were grown in a growth chamber with day and night temperatures of 27 and 21 C, respectively. The light intensity was about 2,000 ft-c during a 14-hr photoperiod. The tomato plants were grown in a greenhouse at ambient daytime temperatures of 16 to 35 C, and 27 C at night.

The plants were inoculated by the injection-infiltration method (3). Portions of the primary leaves of 12-day-old bean plants were inoculated by injecting a bacterial suspension into the leaf tissues with a 26-gauge hypodermic needle. Injection was usually made near the center of the midrib on the undersurface of the leaf so that a water-soaked area occurred on both sides of the midrib and provided sufficient area for sampling. Usually, one of the primary leaves was inoculated with one bacterial culture, and the other primary leaf, with another culture. The inoculated bean plants were returned immediately to the growth chamber. The fourth leaf from the base of tomato plants about 50 days old was inoculated. The leaves below the fourth leaf were removed. The leaflets of the fourth leaf were also removed, except the terminal and the four largest expanded side-leaflets. Usually, the leaflets on one side of the leaf were inoculated with one bacterial culture, and the opposite leaflets with another culture, as described above. The inoculated tomato plants were placed in the growth chamber used for growing bean plants.

The number of viable bacterial cells in the leaf tissues was determined by dilution plating. The inoculated leaves were washed thoroughly with distilled water and rinsed with sterile distilled water. Twenty leaf discs, two from each leaf or leaflet, were cut from the inoculated areas with a cork borer (6 mm diam) as soon as possible (0 days), and then 1, 2, 3, 4, 5, 6, 8, 10, 12, and 14 days after inoculation. The discs were ground with 2 ml sterile distilled water in a sterile tissue grinder, and dilutions were prepared. Samples (0.1 ml) of the appropriate dilutions were placed in sterilized petri plates and mixed thoroughly with about 10 ml of melted (47 C) Difco nutrient agar plus 0.5% glucose and 0.5% yeast extract. Three plates were prepared for each sample. After 3 to 5 days at 27 C, colonies were counted, and the number of viable bacteria per leaf disc (6 mm diam) was calculated.

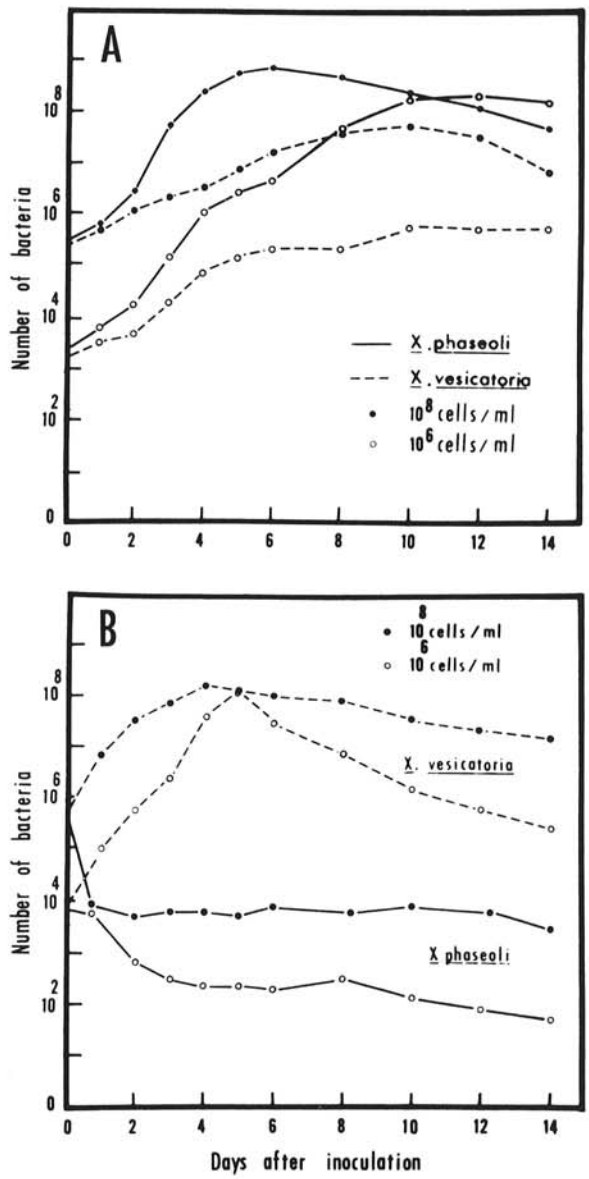

Fig. 1. Number of bacteria recovered from 6-mm diam discs of leaf tissue of **A)** bean **B)** tomato at intervals after inoculation by injection-infiltration with 10^8 or 10^6 cells/ml of *Xanthomonas phaseoli* or *X. vesicatoria*. Each reading represents the average for 20 discs.

RESULTS.—*Multiplication and symptom development in bean leaves.*—Results of a typical experiment concerning the growth patterns of *X. phaseoli* and *X. vesicatoria* in bean leaves are illustrated in Fig. 1-A. Both pathogens multiplied in the leaves. Growth of *X. phaseoli*, however, was more rapid than that of *X. vesicatoria*. When the inoculum

contained 10^8 cells/ml, populations of *X. phaseoli* increased until 6 days after inoculation, then decreased gradually, whereas the number of cells of *X. vesicatoria* reached a maximum after 10 days. The maximal number of viable cells of *X. phaseoli* recovered from the inoculated leaves was about 25 times greater than that of *X. vesicatoria*. When the inoculum contained 10^6 cells/ml, the population of *X. phaseoli* was greatest at 10 days after inoculation, whereas the population of *X. vesicatoria* increased initially, but remained more or less constant between 5 to 14 days after inoculation. At the time of maximal population, there were about 600-fold more cells of *X. phaseoli* than of *X. vesicatoria*.

Necrosis of the infiltrated areas of the leaves was caused by both pathogens when the inoculum concentration was 10^8 cells/ml (Fig. 2-A). The necrotic symptoms always appeared when populations of the bacteria were at their maxima in leaves; i.e., tissue necrosis occurred at 6 and 10 days after inoculation with *X. phaseoli* and *X. vesicatoria*, respectively. The symptoms produced by the two pathogens were different. Yellowish discoloration in the inoculated areas, which appeared at 3 days after inoculation, was the first symptom produced by *X. phaseoli*. This was followed by the appearance of water-soaking on the 5th day, and a general necrosis of the inoculated areas on the 6th day. After 14 days, a yellow chlorosis had developed in the areas which surrounded the inoculated portions of the leaves. *Xanthomonas vesicatoria* also induced a yellowish discoloration by 3 days after inoculation, but the yellowing became progressively more intense, and finally the areas became brown and necrotic by 10 days after inoculation. However, *X. vesicatoria* did not cause the appearance of water-soaking or the expansion of the symptom outside the infiltrated areas.

When the inoculum contained 10^6 cells/ml, *X. vesicatoria* did not produce any visible symptoms, whereas a necrosis developed 10 days after inoculation with *X. phaseoli*. The necrosis was preceded by the appearance of numerous water-soaked spots in the inoculated areas 6 or 7 days after inoculation.

Multiplication and symptom development in tomato leaves.—Population trends of the bacteria in tomato leaves are shown in Fig. 1-B. A striking difference in growth response between these two pathogens was observed. The populations of the tomato pathogen, *X. vesicatoria*, increased rapidly for 4 and 5 days after inoculation with inoculum concentrations of 10^8 and 10^6 cells/ml, respectively, then gradually decreased. The population of viable cells of the bean pathogen, *X. phaseoli*, declined very sharply after inoculation with either concentration of inoculum; the population then remained nearly stable, or decreased only slightly until the termination of the experiment (14 days).

A typical hypersensitive response of tomato leaves to *X. phaseoli* occurred when the leaves were inoculated with 10^8 cells/ml of the bacterium (Fig. 2-B, b). Within 24 hr after inoculation, the inoculated areas of leaves collapsed, became dehydrated, and

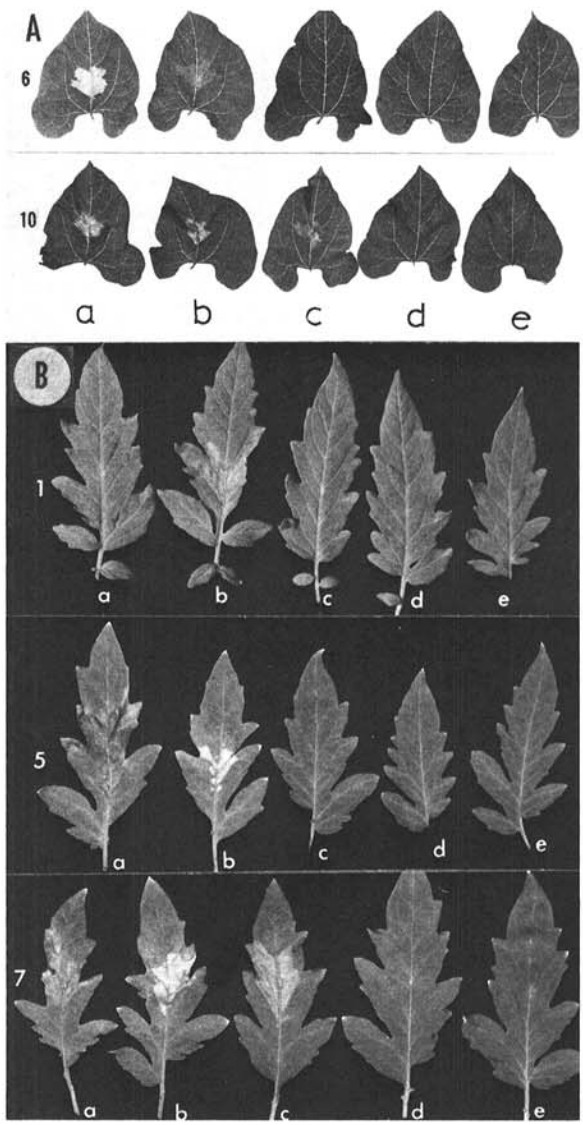

Fig. 2. A) Symptoms produced on bean leaves 6 and 10 days after injection-infiltration of (a) 10^8 cells/ml of *Xanthomonas phaseoli*; (b) 10^8 cells/ml of *X. vesicatoria;* (c) 10^6 cells/ml of *X. phaseoli*; (d) 10^6 cells/ml of *X. vesicatoria*; and (e) sterile distilled water. **B)** Symptoms produced on tomato leaves 1, 5, and 7 days after injection-infiltration of (a) 10^8 cells/ml of *X. vesicatoria*; (b) 10^8 cells/ml of *X. phaseoli*; (c) 10^6 cells/ml of *X. vesicatoria*; (d) 10^6 cells/ml of *X. phaseoli*; and (e) sterile distilled water.

soon developed a light-brown necrosis. Inoculation with 10^6 cells/ml of *X. phaseoli*, however, did not produce visible symptoms on the leaves (Fig. 2-B, d). Symptoms produced by *X. vesicatoria* at 10^8 cells/ml did not appear until 3 days after inoculation when the inoculated portions showed a slight chlorosis and water-soaking. The inoculated tissues became necrotic by 4 days after inoculation (Fig. 2-B, a). Symptoms produced by inoculation with 10^6 cells/ml of *X. vesicatoria* were similar to those described for a concentration of 10^8 cells/ml except that there was a

delay of about 2 days in the appearance of symptoms (Fig. 2-B, c).

DISCUSSION.—Comparative studies on growth of *X. phaseoli* and *X. vesicatoria* in bean and in tomato leaves showed that the pathogens multiplied more rapidly in their natural host than in the nonhost plants. These results are in agreement with those reported for other host-pathogen combinations (1, 2, 4, 6). However, the population of *X. vesicatoria*, nonpathogenic to bean, increased slowly in bean leaves (Fig. 1-A), while the population of *X. phaseoli*, nonpathogenic to tomato, declined in tomato leaves after inoculation (Fig. 1-B). The fact that the numbers of cells of *X. phaseoli* did not increase in the tomato plants differs from most of the reports for other incompatible host-pathogen relationships (5), but it supports the observation of Lozano & Sequeira (7) that not all incompatible bacterial plant pathogens demonstrate an initial increase in numbers.

In our study, the development of symptoms differed for each incompatible host-pathogen relationship. Leaves became necrotic when inoculated with 10^8 but not with 10^6 cells/ml of either bacterium. *Xanthomonas phaseoli* induced rapid development of a typical hypersensitive reaction in tomato leaves, whereas *X. vesicatoria* in bean leaves caused a slow development of a necrotic symptom which was preceded by development of a yellowish discoloration. It appears, therefore, that the results obtained for one incompatible host-pathogen combination are not necessarily the same as the results for other incompatible combinations.

The necrotic symptom produced in bean leaves by *X. vesicatoria* at 10^8 cells/ml raises the question of whether the necrotic reaction observed was similar to the hypersensitive reaction described by Klement & Goodman (5). The typical hypersensitive response of plants to incompatible pathogens is usually characterized by rapid death of infected tissues, and necrosis always appears earlier than the typical symptoms caused by the compatible pathogens (5). However, as reported herein, necrosis induced in bean leaves by the incompatible pathogen, *X. vesicatoria*, occurred 4 days later than the appearance of necrosis induced by the compatible pathogen, *X. phaseoli* (Fig. 2-A). The abrupt termination of a rapid initial increase in population, which is characteristic of many pathogens in nonhost plants, was not observed for *X. vesicatoria* in bean leaves (Fig. 1-A). This observation may explain why rapid development of a necrotic reaction did not occur. The delayed appearance of necrosis also might be due to the experimental conditions used, because it has been shown that environmental factors, particularly light and temperature, greatly influence the development of the hypersensitive reaction (5, 7). However, when using the same experimental conditions, the hypersensitive reaction occurred in tomato leaves inoculated with the incompatible pathogen, *X. phaseoli*. It also has been observed that *Xanthomonas campestris*, the cabbage pathogen, induced rapid necrosis in tomato leaves, but slow necrosis in bean leaves (S.-T. Hsu, *unpublished data*). According to Lozano & Sequeira (7), the hypersensitive reaction did not occur in any incompatible host-pathogen combination. They also reported that *Xanthomonas axonopodis* caused only a slight yellowing in the inoculated tobacco leaves. This result was very similar to the reaction of bean leaves to *X. vesicatoria* as reported herein. Whether a similar necrotic reaction would develop in tobacco leaves inoculated with *X. axonopodis* is not known, since the observations by Lozano & Sequeira (7) were made only at 48 hr after inoculation.

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