Interaction between Xanthomonas phaseoli, Xanthomonas vesicatoria, Xanthomonas campestris, and Pseudomonas fluorescens in Bean and Tomato Leaves

Shih-Tien Hsu and Robert S. Dickey

Graduate Student and Professor, respectively, Department of Plant Pathology, Cornell University, Ithaca, New York 14850. Present address of senior author: Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, Republic of China.

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ABSTRACT

Streptomycin-resistant mutants were used to differentiate between two bacterial species growing together in the same leaf tissue after inoculation by the injection-infiltration method. In bean leaves, prior inoculation (4 days) with *Xanthomonas phaseoli* stimulated the growth of *Xanthomonas vesicatoria* and *Xanthomonas campestris,* but not *Pseudomonas fluorescens.* The population of *X. phaseoli* was decreased, whereas the population of *X. vesicatoria* was increased when bean leaves were inoculated simultaneously with mixtures of the two pathogens; prior inoculation (4 days) with *X. vesicatoria, X. campestris,* or *P. fluorescens* reduced the growth of *X. phaseoli.* Similarly, in tomato leaves, prior inoculation (2 days) with *X. vesicatoria* enhanced the growth of *X. phaseoli* and *X. campestris,* but not *P. fluorescens.* The population of *X. vesicatoria* was decreased, whereas the population of *X. phaseoli* was increased when tomato leaves were inoculated simultaneously with the mixtures of the two pathogens; prior inoculation (2 days) with *X. phaseoli, X. campestris,* or *P. fluorescens* reduced the growth of *X. vesicatoria.*

Prior inoculation of bean leaves with *P. fluorescens* and of tomato leaves with *P. fluorescens* or *X. phaseoli* delayed the development of symptoms caused by *X. phaseoli* and *X. vesicatoria,* respectively.

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The influence of interactions between pathogenic and nonpathogenic bacteria or between different strains of a pathogenic bacterium on disease development in plants has recently been studied (1, 2, 5, 8, 18, 20). Prior inoculation of plants with avirulent mutants or heat-killed bacteria may protect a plant for limited or sustained periods against the virulent pathogen (4, 7, 9, 14, 16). Extracts from tobacco leaves inoculated with the incompatible race 2 of *Pseudomonas solanacearum* E. F. Smith inhibit the growth of both compatible race 1 and incompatible race 2 of the pathogen (15). However, the effect of a compatible pathogen on development of incompatible bacteria in plants has received little attention. The effect of the interactions between bacteria in bean and in tomato leaves is reported herein.

MATERIALS AND METHODS.—*Xanthomonas phaseoli* (isolate XP-24), *X. vesicatoria* (isolate XV-21), *Xanthomonas campestris* (isolate XC-3), and an isolate of *Pseudomonas fluorescens* were used in these studies. These cultures were single-cell isolates (6), or originated from single colonies of a dilution series. A streptomycin-resistant mutant of each culture was isolated by a plating of 0.1 ml of each bacterial suspension (ca. 10^9 cells/ml) on Difco nutrient agar plus 0.5% glucose and 0.5% yeast extract (NAGY) containing 250 μg streptomycin sulfate/ml. The frequency of resistant cells was less than $10/10^9$ cells. These one-step mutants were resistant to at least 1,000 μg streptomycin sulfate/ml. The pathogenicity of the *Xanthomonas* cultures and their streptomycin-resistant mutants was confirmed by inoculation of their natural hosts, bean (*Phaseolus vulgaris* L. 'Red Kidney') (3), tomato (*Lycopersicon esculentum* Mill. 'Valiant') (17), or cauliflower (*Brassica oleracea* L. var. *botrytis* L. 'Super Snowball') (19). Streptomycin resistance was used as a marker for differentiation between two different bacteria when they were present together in the plants. In preliminary tests, changes in populations of the cultures and their respective streptomycin-resistant mutants in the leaves were similar, and colony counts for each streptomycin-resistant mutant were comparable when NAGY and NAGY plus streptomycin sulfate were used for the dilution series. The *Xanthomonas* sp. were maintained on potato-dextrose agar (pH 7.0) which contained 1.0% dextrose; *P. fluorescens* was kept on Difco nutrient agar, and all cultures were stored at 5 C.

The procedures for preparation of the bacterial suspensions, growth of bean and tomato plants used for inoculation, inoculation of the plants, and determination of viable bacteria in the inoculated leaves were the same as those described previously (11).

RESULTS.—*Bacterial populations and symptom development in leaves inoculated simultaneously with mixtures of X. phaseoli and X. vesicatoria.*—Bean leaves were inoculated by injection-infiltration with suspensions that contained a uniform number of cells of the streptomycin-resistant mutant of *X. phaseoli* but different numbers of *X. vesicatoria.* The inocula were prepared by mixing suspensions of both bacterial pathogens just before inoculation. The ratios of *X. phaseoli* to *X. vesicatoria* in inocula were 100:0, 100:25, 100:50, 100:75, and 100:100; ratios of 0:25, 0:50, 0:75, and 0:100 were used for controls. The value, 100, was equivalent to ca. 5×10^7 cells/ml for each pathogen. The bacterial populations in the inoculated leaves were determined 6 days after inoculation. The appropriate dilution series were plated

on both NAGY and NAGY plus 500 μg streptomycin sulfate/ml. The number of cells of *X. phaseoli* was calculated from the colony counts made with NAGY plus streptomycin sulfate, whereas the differences in colony counts with NAGY and with NAGY plus streptomycin sulfate were attributed to the number of cells of *X. vesicatoria*. A progressive increase in the concentration of *X. vesicatoria* in the inoculum containing both pathogens caused a progressive decrease in the numbers of *X. phaseoli*, and tended to decrease the numbers of *X. vesicatoria* isolated from leaf tissue (Table 1). The numbers of *X. vesicatoria* progressively increased with an increase in the concentration of the pathogen in the inoculum containing only *X. vesicatoria*, but they never were as large as when the inoculum contained both pathogens.

Tomato leaves were similarly infiltrated as described for bean leaves, except that the value, 100, was equivalent to ca. 5×10^5 cells/ml in the suspension. The bacterial populations were determined 4 days after inoculation. The numbers of *X. vesicatoria* were progressively decreased with an increase in the number of cells of *X. phaseoli* in the inoculum (Table 2). The numbers of *X. phaseoli* were greater when the bacterium was associated with *X. vesicatoria*.

Severity and development of symptoms were not appreciably affected by the simultaneous inoculation with the two pathogens. Necrosis had developed in bean leaves 6 days after inoculation with mixtures of *X. phaseoli* and *X. vesicatoria* or with *X. phaseoli* alone, whereas *X. vesicatoria* alone had produced only a slightly yellow discoloration of the infiltrated areas of the leaves. Necrosis appeared 7 days after inoculation of tomato leaves with the mixtures or with *X. vesicatoria* alone. The tomato leaves inoculated with *X. phaseoli* alone showed no symptoms.

Effect of prior inoculation with X. phaseoli on growth of X. vesicatoria, X. campestris, or P. fluorescens and on symptom development in bean leaves.— Bean leaves either were inoculated by injection-infiltration with a suspension containing 10^6 cells/ml of *X. phaseoli*, were infiltrated with sterile distilled water, or were not inoculated. Four days later, portions of the previously inoculated or water-infiltrated areas either were (i) inoculated with *X. vesicatoria*, *X. campestris*, or *P. fluorescens* (10^6 cells/ml); (ii) infiltrated with sterile distilled water; or (iii) were not treated. The previously noninoculated leaves also were inoculated with *X. vesicatoria*. Bacterial populations in the leaves were determined at 2 hr and 2, 4, and 6 days after the second inoculation or treatment. The samples used for determination of the population were obtained from the areas of the leaves that had been treated or inoculated.

Populations of *X. vesicatoria* were comparable in leaves which were either infiltrated with water or were not infiltrated 4 days prior to inoculation with *X. vesicatoria*. However, growth of *X. vesicatoria* was markedly stimulated in leaves inoculated 4 days previously with *X. phaseoli* (Fig. 1-D, E). Populations of *X. phaseoli* in leaves infiltrated with *X. vesicatoria* or with sterile distilled water 4 days after inoculation with *X. phaseoli* (Fig. 1-A, B) were similar, and were greater than those without infiltration (Fig. 1-C). This indicates that the greater increase in numbers of *X. phaseoli* following the second infiltration was primarily due to the water rather than to *X. vesicatoria*.

Growth of *X. campestris* was enhanced when the leaves were previously inoculated with *X. phaseoli*, although *X. campestris* alone was able to increase in numbers. In contrast, prior inoculation of leaves with *X. phaseoli* had no effect on the growth of *P. fluorescens*. Populations of *P. fluorescens*, whether alone or in association with *X. phaseoli*, declined after inoculation. Populations of *X. phaseoli* were greater in leaves which later were inoculated with *X. campestris* or *P. fluorescens*, or later were infiltrated with water than in leaves which did not receive any further treatments.

When bean leaves were inoculated with *X. phaseoli*

TABLE 1. Number of viable cells of *Xanthomonas phaseoli* and *Xanthomonas vesicatoria* in bean leaves 6 days after inoculation with mixtures of cells of *X. phaseoli* and *X. vesicatoria*

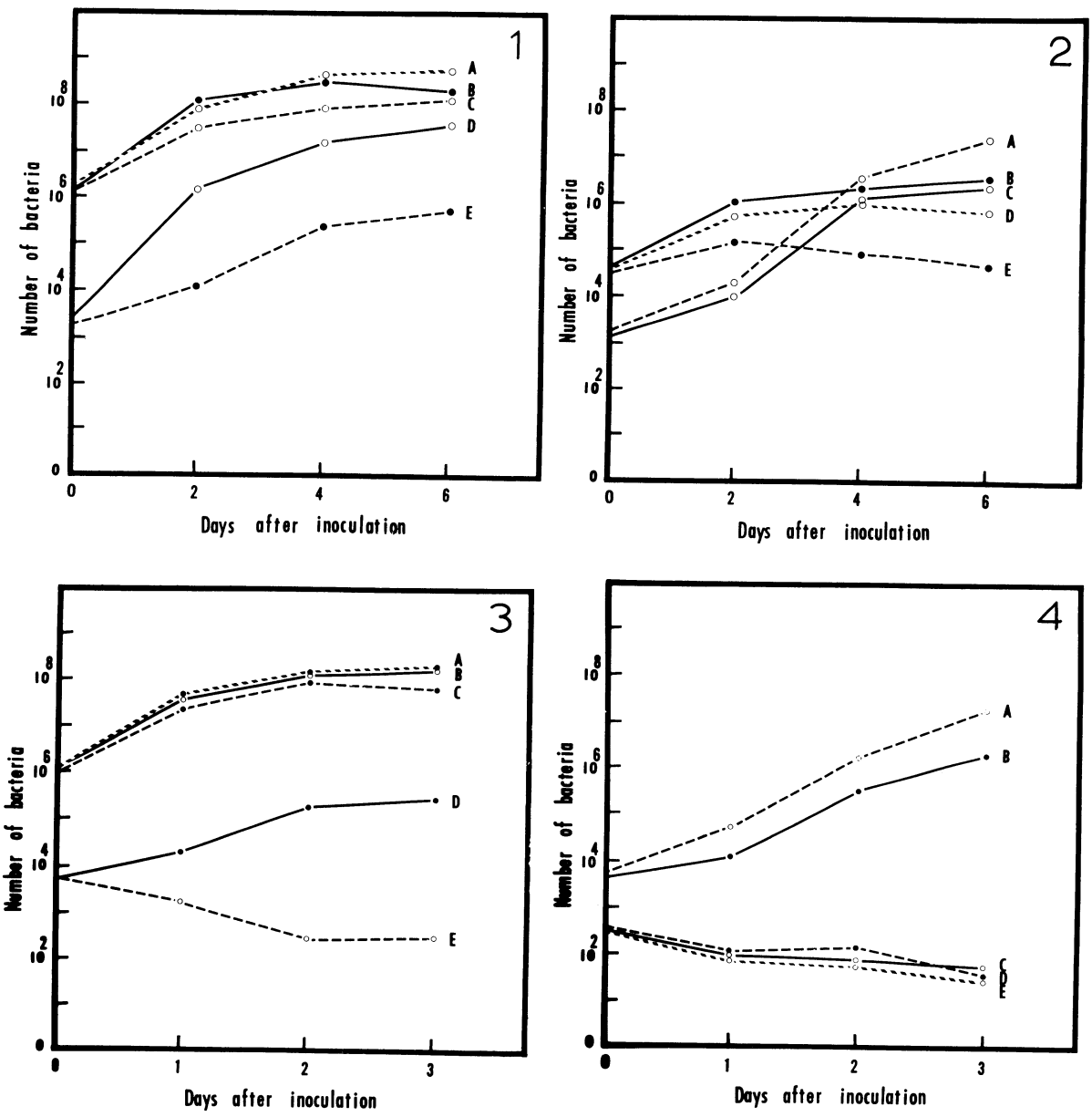
Ratio of cells of *X. phaseoli* to cells of *X. vesicatoria* in the inoculum[a]	No. bacteria/6-mm-diam disc of leaf tissue[b]	
	X. phaseoli ($\times 10^7$)	*X. vesicatoria* ($\times 10^5$)
100:0	29.2	0.0
100:25	25.6	737.0
100:50	19.7	675.0
100:75	9.0	431.0
100:100	7.2	450.0
0:25	0.0	1.9
0:50	0.0	15.9
0:75	0.0	73.3
0:100	0.0	196.0

[a] 100 = approximately 5×10^7 cells/ml.
[b] Each value represents the average for 20 leaf discs.

TABLE 2. Number of viable cells of *Xanthomonas vesicatoria* and *Xanthomonas phaseoli* in tomato leaves 4 days after inoculation with mixtures of cells of *X. vesicatoria* and *X. phaseoli*

Ratio of cells of *X. vesicatoria* to cells of *X. phaseoli* in the inoculum[a]	No. bacteria/6-mm-diam disc of leaf tissue[b]	
	X. vesicatoria ($\times 10^6$)	*X. phaseoli* ($\times 10^1$)
100:0	35.6	0.0
100:25	21.2	270.0
100:50	11.5	720.0
100:75	7.4	1,160.0
100:100	2.3	1,300.0
0:25	0.0	8.1
0:50	0.0	9.0
0:75	0.0	12.6
0:100	0.0	14.5

[a] 100 = approximately 5×10^5 cells/ml.
[b] Each value represents the average for 20 leaf discs.



and 4 days later infiltrated with *X. vesicatoria, X. campestris,* or water, necrosis occurred 8 days after inoculation with *X. phaseoli* in the areas in which the two treatments coincided, but only water-soaked spots developed in the remainder of inoculated areas which were not infiltrated by the second treatment. At the same time, water-soaking was also observed in leaves which were inoculated with *X. phaseoli,* but which did not receive a second treatment. These differences of types of symptoms may be attributed to the differences in the population of *X. phaseoli* (Fig. 1-A, B, C). However, only water-soaking occurred in leaves inoculated with *X. phaseoli* and 4 days later infiltrated with *P. fluorescens.* This indicates that *P. fluorescens* may have interferred with disease development, since an effect of water was not observed. *Xanthomonas vesicatoria, X. campestris,* or *P. fluorescens* alone had no visible effect on bean leaves.

The effect of prior inoculation with X. vesicatoria on growth of X. phaseoli, X. campestris, or P. fluorescens and symptom development in tomato leaves.—Tomato leaflets either were first inoculated with *X. vesicatoria* (10^6 cells/ml), were infiltrated with sterile distilled water, or were not infiltrated. Two days later, the leaflets were (i) infiltrated with *X. phaseoli, X. campestris,* or *P. fluorescens* (10^6 cells/ml); (ii) with sterile distilled water; or (iii) were not given an additional treatment. Area of the second infiltration almost completely included the area of

Fig. 1-4. 1) Effect of prior inoculation with *Xanthomonas phaseoli* on growth of *Xanthomonas vesicatoria* in bean leaves. *X. vesicatoria* or sterile distilled water was injected-infiltrated into the leaves 4 days after injection-infiltration with *X. phaseoli.* 0 day = 4 days after inoculation with *X. phaseoli.* The number of bacteria = average number of bacteria/disc (6 mm diam) for 20 discs of leaf tissue. Populations of: (A) *X. phaseoli* in leaves which were infiltrated with water 4 days after infiltration with *X. phaseoli;* (B) *X. phaseoli* in leaves which were infiltrated with *X. vesicatoria* 4 days after infiltration with *X. phaseoli;* (C) *X. phaseoli* in leaves which were only infiltrated with *X. phaseoli;* (D) *X. vesicatoria* in leaves which were infiltrated 4 days previously with *X. phaseoli;* and (E) *X. vesicatoria* in leaves which were infiltrated 4 days previously with water. **2)** Effect of prior inoculation with *X. vesicatoria* on growth of *X. phaseoli* in bean leaves. *X. phaseoli* or sterile distilled water was injected-infiltrated into the leaves 4 days after injection-infiltration with *X. vesicatoria.* 0 day = 4 days after injection-infiltration with *X. vesicatoria.* Populations of: (A) *X. phaseoli* in leaves which were infiltrated 4 days previously with water; (B) *X. vesicatoria* in leaves which were infiltrated with *X. phaseoli* 4 days after infiltration with *X. vesicatoria;* (C) *X. phaseoli* in leaves which were infiltrated 4 days previously with *X. vesicatoria;* (D) *X. vesicatoria* in leaves which were infiltrated with water 4 days after infiltration with *X. vesicatoria;* and (E) *X. vesicatoria* in leaves which were only infiltrated with *X. vesicatoria.* **3)** Effect of prior inoculation with *X. vesicatoria* on growth of *X. phaseoli* in tomato leaves. *X. phaseoli* or sterile distilled water was injected-infiltrated into the leaves 2 days after injection-infiltration with *X. vesicatoria.* 0 day = 2 days after infiltration with *X. vesicatoria.* Populations of: (A) *X. vesicatoria* in leaves which were infiltrated with water 2 days after infiltration with *X. vesicatoria;* (B) *X. vesicatoria* in leaves which were infiltrated with *X. phaseoli* 2 days after infiltration with *X. vesicatoria;* (C) *X. vesicatoria* in leaves which were only infiltrated with *X. vesicatoria;* (D) *X. phaseoli* in leaves which were infiltrated 2 days previously with *X. vesicatoria;* and (E) *X. phaseoli* in leaves which were infiltrated with water 2 days prior to inoculation with *X. phaseoli.* **4)** Effect of prior inoculation with *X. phaseoli* on growth of *X. vesicatoria* in tomato leaves. *X. vesicatoria* or sterile distilled water was injected-infiltrated into the leaves 2 days after injection-infiltration with *X. phaseoli.* 0 day = 2 days after infiltration with *X. phaseoli.* Populations of: (A) *X. vesicatoria* in leaves which were infiltrated with water 2 days prior to inoculation with *X. vesicatoria;* (B) *X. vesicatoria* in leaves which were infiltrated 2 days previously with *X. phaseoli;* (C) *X. phaseoli* in leaves which were infiltrated with *X. vesicatoria* 2 days after infiltration with *X. phaseoli;* (D) *X. phaseoli* in leaves which were only infiltrated with *X. phaseoli;* and (E) *X. phaseoli* in leaves which were infiltrated with water 2 days after infiltration with *X. phaseoli.*

the first infiltration. Bacterial populations were determined at 2 hr and 1, 2, and 3 days after the second inoculation or treatment.

Populations of *X. phaseoli* alone did not increase in tomato leaves (Fig. 3-E), which agrees with the results reported previously (11). Populations of *X. phaseoli* increased in leaves which had been infiltrated with *X. vesicatoria* 2 days previously (Fig. 3-D). *X. vesicatoria* increased similarly in leaves that were infiltrated with water, *X. phaseoli,* or *X. campestris,* or were not infiltrated 2 days after inoculation with *X. vesicatoria* (Fig. 3-A, B, C), but increased more slowly when infiltrated with *P. fluorescens.*

Populations of *X. campestris* alone slowly increased in tomato leaves, but growth was enhanced when the leaves were previously infiltrated with *X. vesicatoria.* Populations of *P. fluorescens* declined although the decrease in population was slower in leaves that had been previously inoculated with *X. vesicatoria.*

Infiltration with *X. phaseoli, X. campestris, P. fluorescens,* or water 2 days after inoculation with *X. vesicatoria* did not affect development of symptoms by *X. vesicatoria* in tomato leaves. Slight yellowing and partial necrosis appeared 5 days after inoculation with *X. vesicatoria,* and the entire inoculated areas became necrotic by the 6th day. *X. phaseoli, X. campestris,* or *P. fluorescens* alone did not produce any visible symptoms.

Effect of prior inoculation with X. vesicatoria, X. campestris, or P. fluorescens on growth of X. phaseoli and symptom development in bean leaves.—Prior infiltration with *X. vesicatoria* reduced the growth of *X. phaseoli* (Fig. 2-A, C). However, the growth of *X. vesicatoria* was greater in leaves which were first inoculated with *X. vesicatoria* and 4 days later infiltrated with *X. phaseoli* or with water than in comparable leaves which were not given the additional treatment (Fig. 2-B, D, E). The results of prior inoculation with *X. campestris* were similar to those for *X. vesicatoria.* Prior inoculation with *P. fluorescens* also caused reduction in the growth of *X. phaseoli,* but the population of *P. fluorescens* gradually declined after inoculation, regardless of the treatment.

Although growth of *X. phaseoli* was reduced by the prior inoculation with *X. vesicatoria* or with *X. campestris,* the effect of these pathogens on symptoms produced by *X. phaseoli* was not easily determined. The leaf areas that were preinfiltrated with *X. vesicatoria* or *X. campestris* developed necrosis at 4 days after being infiltrated with *X. phaseoli* or with water. Prior infiltration with *P. fluorescens,* however, caused a delay of 4 to 5 days in the appearance of necrotic lesions produced by *X. phaseoli.*

Effect of prior inoculation with X. phaseoli, X. campestris, or P. fluorescens on growth of X. vesicatoria and symptom development in tomato leaves.—The population of *X. vesicatoria* increased more rapidly in leaves which were previously infiltrated with water than with *X. phaseoli* (Fig. 4-A, B). The populations of *X. phaseoli* declined similarly for all treatments (Fig. 4-C, D, E).

A reduction in the population of *X. vesicatoria* also was recorded when the leaves were inoculated with *X. campestris* prior to the inoculation with *X. vesicatoria.* Infiltration with *X. vesicatoria* or with water in leaves previously inoculated with *X. campestris* slightly increased the populations of *X. campestris* above those in leaves which were inoculated with *X. campestris* alone. Prior inoculation with *P. fluorescens* caused a marked reduction in the

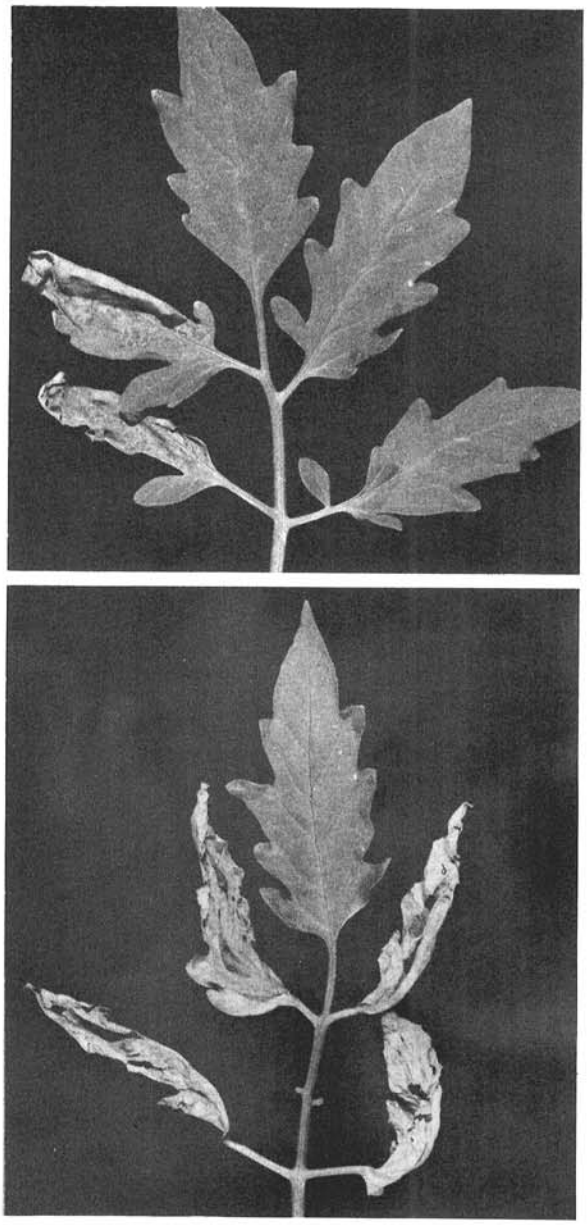


Fig. 5. Effect of prior inoculation with *Xanthomonas phaseoli* on symptoms produced by *Xanthomonas vesicatoria* in tomato leaves 6 (above) and 8 (below) days after inoculation with *X. vesicatoria.* The two leaflets on the left side of the leaf were infiltrated with sterile distilled water, and the two leaflets on the right side of the leaf were infiltrated with 10^6 cells/ml of *X. phaseoli* 2 days prior to the inoculation of all leaflets, except the terminals, with a suspension of 10^6 cells/ml of *X. vesicatoria.*

growth of *X. vesicatoria.* The general decrease in the populations of the saprophyte was similar for all treatments.

The effect of prior inoculation on symptom development of tomato leaves were as follows: (i) Necrosis developed at 6 days after inoculation with *X. vesicatoria* in the leaves which were infiltrated with water and 2 days later infiltrated with *X. vesicatoria,* whereas the appearance of this symptom in leaves previously inoculated with *X. phaseoli* was delayed 2 days (Fig. 5). (ii) Leaves inoculated with *X. phaseoli* and later infiltrated with water or not infiltrated showed no visible symptoms. (iii) Inoculation with *X. campestris* caused a necrosis 2 days after the leaves were subsequently infiltrated with *X. vesicatoria* or water, but the leaves remained symptomless when the leaves were not further infiltrated. (iv) The leaves inoculated with *P. fluorescens*, then later infiltrated with *X. vesicatoria*, did not develop any necrotic symptoms, but yellowing was observed about 10 days after infiltration with *X. vesicatoria.* (v) The leaves inoculated with *P. fluorescens* and later infiltrated with water or not infiltrated showed no symptoms.

DISCUSSION.—The results of this investigation suggest that the growth of both the compatible and the incompatible pathogen in plants is mutually affected, and that the type of interactions depends largely upon the sequence of inoculation with the two pathogens. Several general conclusions may be drawn from the experiments made with bean leaves in which *X. phaseoli* was associated with *X. vesicatoria* or *X. campestris,* and with tomato leaves in which *X. vesicatoria* was interacting with *X. phaseoli* or *X. campestris:* (i) Growth of the compatible pathogen was reduced, but that of the incompatible pathogen was slightly increased or unaffected when leaves were infiltrated first with the incompatible pathogen and later with the compatible bacterium. (ii) The population of the compatible pathogen decreased, but that of the incompatible bacterium increased when leaves were infiltrated simultaneously with a combination of compatible and incompatible pathogens. (iii) Prior inoculation of leaves with a compatible pathogen enhanced growth of an incompatible pathogen subsequently infiltrated, but this enhanced growth of the incompatible pathogen did not significantly change the growth of the compatible pathogen. It also was found that the population of *X. phaseoli* alone was unable to increase in tomato leaves, but it increased in tomato leaves previously infiltrated with *X. vesicatoria* (Fig. 3). The interaction between the saprophyte, *P. fluorescens,* and *X. phaseoli* in bean leaves, or *X. vesicatoria* in tomato leaves, were somewhat different from those discussed above. Whereas prior inoculation of leaves with the saprophyte reduced the growth of a compatible pathogen, prior inoculation of leaves with a compatible pathogen did not enhance the growth of the saprophytic bacterium.

Growth of *X. phaseoli, X. vesicatoria,* and *X. campestris* is inhibited in vitro by *P. fluorescens,* but the species of *Xanthomonas* are not antagonistic to each other (10, 20). It would appear, therefore, that the reduction in growth of the compatible pathogen by its association in leaves with the incompatible pathogen may have been mediated through the plant's metabolism. Perhaps the incompatible pathogen induced a plant defense response which inhibited

the growth of the compatible pathogen, whereas the altered growth of the pathogen in leaves previously inoculated with *P. fluorescens* may have resulted from the direct action of the saprophyte on the pathogen. However, in the latter case, whether the same mechanism observed in vitro also was operative in vivo was not readily established, because the population of the saprophyte steadily declined in bean or in tomato leaves. Moreover, heat-killed cells of *P. fluorescens* have been reported to afford an inhibitory effect toward *P. tabaci* in tobacco leaves (12).

The factors that stimulated the growth of the incompatible pathogen in leaves previously inoculated with the compatible pathogen are not known. The enhanced growth of the incompatible pathogen may be due to the neutralization of the plant defense mechanism by the compatible pathogen. Availability of nutrients at an infection site may also be a factor. Permeability alterations in bean and tomato leaves, as estimated by the elution of radioactive substances from the inoculated leaf tissues of the plants treated with $K_2H^{32}PO_4$ solution, indicated that a compatible pathogen caused a greater change in cell permeability than did an incompatible pathogen (10). If the alteration of cell permeability results in providing available nutrients to the pathogen, as suggested by some workers (13, 21, 22), then the enhanced growth of the incompatible pathogen in leaves previously inoculated with the compatible pathogen may be interpreted as resulting from activities by the compatible pathogen making more nutrients available to the incompatible pathogen. The relationship of availability of nutrients in leaves to enhanced growth of an incompatible pathogen is apparently supported by the observation that growth of *X. vesicatoria* was markedly increased in bean leaves inoculated with cells of the bacterium suspended in a nutrient solution; a similar result also was observed for tomato leaves inoculated with *X. phaseoli,* but to a lesser degree (10). This interpretation, however, did not seem to be true for the saprophyte, since *P. fluorescens* did not multiply in the leaves regardless of whether they were previously inoculated with the compatible pathogen. Some other factors, therefore, probably were responsible for the inability of the saprophyte to grow in bean or in tomato leaves.

The results showed that infiltration of tomato leaves with *X. phaseoli* or with *P. fluorescens* prior to their inoculation with *X. vesicatoria* delayed or reduced disease development caused by *X. vesicatoria.* The delay of symptoms caused by *X. vesicatoria* due to prior inoculation with *X. phaseoli* may reflect the slower rate of growth of *X. vesicatoria* (Fig. 4-A, B) and the subsequent delay in the time required for the population of *X. vesicatoria* to reach the maximum when necrosis is observed (11). However, the effect of prior inoculation of tomato leaves with *X. campestris* on symptoms induced by *X. vesicatoria* and of prior inoculation of bean leaves with *X. vesicatoria,* or with *X. campestris* on symptoms induced by *X. phaseoli,* was not readily explained. The areas of the leaves which were previously infiltrated with the incompatible pathogen developed a necrosis after being infiltrated with the compatible pathogen or with water. The necrosis may have resulted from a hypersensitive response of the leaves to the incompatible pathogen due to an increase in the population of the incompatible pathogen that was sufficient to induce the hypersensitive reaction. The possibility of further distribution of previously infiltrated bacteria by subsequent infiltrations must not be ignored for these types of investigations.

The results of this study again emphasize the fact that precaution must be exercised in preparation of general statements concerning the interaction of bacteria within plants. The results also demonstrate that bacterial species, sequence of inoculation, method of inoculation, and plant species can influence the effects of the interactions.

LITERATURE CITED

1. AVERRE, C. W., III, & A. KELMAN. 1964. Severity of bacterial wilt as influenced by ratio of virulent to avirulent cells of Pseudomonas solanacearum in inoculum. Phytopathology 54:779-783.
2. BRATHWAITE, C. W. D., & R. S. DICKEY. 1971. Role of cellular permeability alterations and pectic and cellulolytic enzymes in the maceration of carnation tissue by Pseudomonas caryophylli and Corynebacterium sp. Phytopathology 61:476-483.
3. BURKHOLDER, W. H. 1930. The bacterial diseases of the bean: a comparative study. Cornell Univ. Agr. Exp. Sta. Mem. 127. 88 p.
4. CARROLL, R. B., & F. L. LUKEZIC. 1971. Induced resistance in alfalfa to Corynebacterium insidiosum by prior treatment with avirulent cells. Phytopathology 61:887 (Abstr.).
5. CROSSE, J. E. 1965. Bacterial canker of stone fruits. VI. Inhibition of leaf scar infection of cherry by a saprophytic bacterium from the leaf surfaces. Ann. Appl. Biol. 56:149-160.
6. DE VAY, J. E., & W. C. SCHNATHORST. 1963. Single-cell isolation and preservation of bacterial cultures. Nature 199:775-777.
7. ERCOLANI, G. L. 1970. Bacterial canker of tomato. IV. The interaction between virulent and avirulent strains of Corynebacterium michiganense (E. F. Sm.) Jens. in vivo. Phytopathol. Mediterranea 9:151-159.
8. FARABEE, G. J., & J. L. LOCKWOOD. 1958. Inhibition of Erwinia amylovora by Bacterium sp. isolated from fire blight cankers. Phytopathology 48:209-211.
9. GOODMAN, R. N. 1967. Protection of apple stem tissue against Erwinia amylovora infection by avirulent strains and other bacterial species. Phytopathology 57:22-24.
10. HSU, S. T. 1970. Comparative growth of Xanthomonas phaseoli and Xanthomonas vesicatoria in bean and tomato leaves and some physiological alterations in the inoculated leaves. Ph.D. Thesis, Cornell Univ., Ithaca, N. Y. 103 p.
11. HSU, S. T., & R. S. DICKEY. 1972. Comparative growth of Xanthomonas phaseoli and Xanthomonas vesicatoria and development of symptoms in bean and tomato leaves. Phytopathology 62:329-332.
12. KLEMENT, Z., & R. N. GOODMAN. 1967. Hypersensitive reaction to infection by bacterial plant pathogens. Annu. Rev. Phytopathol. 5:17-44.
13. LINK, S. K., & H. W. WILCOX. 1936. Relation of nitrogen-carbohydrate nutrition of Stayman apple

trees to susceptibility to fire blight. Phytopathology 26:643-655.
14. LOVREKOVICH, L., & G. L. FARKAS. 1965. Induced protection against wildfire disease in tobacco leaves treated with heat-killed bacteria. Nature 205:823-824.
15. LOZANO, J. C., & L. SEQUEIRA. 1970. Differentiation of races of Pseudomonas solanacearum by a leaf infiltration technique. Phytopathology 60:833-838.
16. MAIN, C. E. 1968. Induced resistance to bacterial wilt in susceptible tobacco cuttings pretreated with avirulent mutants of Pseudomonas solanacearum. Phytopathology 58:1058-1059 (Abstr.).
17. NAYUDU, M. V., & J. C. WALKER. 1960. Bacterial spot of tomato as influenced by temperature and by age and nutrition of the host. Phytopathology 50:360-364.
18. OMER, M. E. H., & R. K. S. WOOD. 1969. Growth of Pseudomonas phaseolicola in susceptible and resistant bean plants. Ann. Appl. Biol. 63:103-116.
19. SMITH, E. F. 1911. Bacteria in relation to plant diseases, p. 300-334. Vol. II. Carnegie Institution, Washington, D. C.
20. TELIZ-ORTIZ, M., & W. H. BURKHOLDER. 1960. A strain of Pseudomonas fluorescens antagonistic to Pseudomonas phaseolicola and other bacterial plant pathogens. Phytopathology 50:119-123.
21. THATCHER, F. S. 1939. Osmotic and permeability relations in the nutrition of fungus parasites. Amer. J. Bot. 26:449-458.
22. WILLIAMS, P. H., & N. T. KEEN. 1967. Relation of cell permeability alterations to water congestion in cucumber angular leaf spot. Phytopathology 57:1378-1385.