Pathogenesis as Influenced by the Interaction of Two Virulent Strains of Pseudomonas solanacearum in Inoculated Tobacco Plants

Eldon I. Zehr

Former Graduate Assistant, Department of Plant Pathology, Cornell University, Ithaca, New York 14850. Present address: Department of Plant Pathology and Physiology, Clemson University, Clemson, South Carolina 29631.

The writer thanks K. G. Parker, R. S. Dickey, and H. D. Thurston for advice and interest.

Supported in part by Ford Foundation funds under the University of The Philippines-Cornell Graduate Education Program. Portion of a Ph.D. thesis.

Accepted for publication 18 March 1971.

ABSTRACT

Tobacco plants were stem-inoculated with two Philippine isolates of *Pseudomonas solanacearum* virulent to tomato, but differing in virulence to tobacco. When the plants were inoculated near the shoot tip with mixtures of the two isolates, wilt was less severe than when tobacco plants were inoculated with the same number of cells of the more virulent isolate alone. The effect was most evident when the mixture contained a high percentage of cells of low virulence to tobacco, but was apparent even when the mixture contained equal numbers of cells of each isolate. However, when older plant tissues (stems) were inoculated with any proportion, disease severity was not reduced. When plants were inoculated near the shoot tip with the isolate of low virulence and in the lower stem with the more virulent isolate, wilt was less severe, but when the isolates were reversed in position, no such effect was observed. Phytopathology 61:987-989.

Strains of *Pseudomonas solanacearum* that are highly virulent to tomato (*Lycopersicon esculentum* Mill.), but not to tobacco (*Nicotiana tabacum* L.), are common in The Philippines, even in localities where tobacco is an important crop (9). Nevertheless, bacterial wilt of tobacco is an important disease, and isolates of *P. solanacearum* from wilted tobacco plants are highly virulent to tobacco. Despite the difference in virulence, strains from tobacco and tomato are similar in cultural, physiological, and biochemical properties, and differ only slightly in virulence to hosts other than tobacco (9, 10).

In tobacco fields of The Philippines, bacterial wilt symptoms appear later and develop more slowly and less uniformly than in other susceptible crops (8). Several possible explanations for the slow rate of disease development are that (i) strains of the pathogen which are present in The Philippines may be less virulent to tobacco than to tomato and other crops; (ii) the strain that is virulent to tobacco may be of low frequency relative to strains that are of low virulence; or (iii) since most tobacco is grown during the dry season, moisture or other environmental factors may not favor rapid wilt development.

The possibility that strains of low virulence to tobacco might be involved in the pattern of wilt development was suggested by two reports. Averre & Kelman (2) reported that avirulent variants of the pathogen can reduce disease severity in normally susceptible tobacco, tomato, or eggplants when the plants are inoculated with mixtures of virulent and avirulent cells. Disease severity is also reduced when tobacco plants are inoculated with virulent cells from banana (race 2), which normally do not attack tobacco. Later, Main (6) observed that inoculation first with avirulent variants and then with virulent cells also resulted in decreased disease severity. The present study was undertaken to determine whether strains from The Philippines that are similar except for virulence to tobacco might also interact in disease development in inoculated tobacco plants.

MATERIALS AND METHODS.—*Pseudomonas solanacearum* isolates from tobacco and tomato (designated I-2 and I-3, respectively, in other studies (9, 10)) were collected near Ilagan, Isabela, The Philippines. Both isolates were highly virulent to tomato, but although the tobacco isolate (VT) was virulent to tobacco, the tomato isolate (AT) was not. In pathogenicity tests (9), both isolates corresponded to race 1 (4), except for the low virulence of AT to tobacco. After purification on tetrazolium medium (TTC) (5), the two isolates were stored in sterile, distilled water at room temperature (23-32 C).

The procedures described by Averre & Kelman (2) were used. Inoculum was prepared from 48-hr-old cultures on TTC medium. Discrete fluidal (virulent) colonies were selected for preparation of the bacterial suspension. Final cell concentrations were 10^9 cells/ml, determined by total cell count according to the Breed method (3). Proportions of 50:50, 5:95, and 0.5:99.5 of VT:AT were used.

Young Reax tobacco plants were grown in sterilized soil and transplanted to 6-inch clay pots. Plants were stem-inoculated with ca. 0.1 ml of inoculum/plant and evaluated for symptom development according to methods described previously (9). Disease severity ratings were converted to a disease index as described by Winstead & Kelman (7). Ten replicates/treatment were used, and the experiment was repeated 3 times; once under a glass roof with open sides under tropical climatic conditions (26-34 C) in The Philippines, and twice in the greenhouse at Ithaca, New York (23-29 C).

The method of Abo-El-Dahab & El-Goorani (1) was used to test for possible antagonism between the two strains in vitro, using their nutrient-glycerol-casein

TABLE 1. Symptom development in Reax tobacco plants inoculated with different cell ratios of an isolate of *Pseudomonas solanacearum* virulent to tomato and tobacco (VT) and an isolate virulent to tomato but not to tobacco (AT)

Conc (cells/ml)		Disease index[a]		
VT	AT	7[b]	14	19
1×10^9	0	66	94	97
5×10^8	0	64	92	98
5×10^7	0	70	100	100
5×10^6	0	44	86	96
5×10^8	5.0×10^8	47	67	76
5×10^7	9.5×10^8	22	40	54
5×10^6	9.95×10^8	44	42	54
0	1.0×10^9	26	40	40
0[c]	0	0	0	0

[a] Disease index computed by the method of Winstead & Kelman (7). 0 = no symptoms; 20 = localized necrosis at site of inoculation; 40 = vascular browning 2 cm or more from site of inoculation; 60 = 1 or more leaves entirely wilted; 80 = all leaves wilted; 100 = plant dead.
[b] Number of days after inoculation.
[c] Controls inoculated with sterile distilled water.

hydrolysate agar (NGCA) medium and TTC medium. Plates of both media were streaked with a single line of aqueous bacterial suspension of isolate AT or VT. Possible antagonism was tested by streaking each isolate perpendicular to the other on the same plate at the time the first streak was made, or after 5 days as described by Abo-El-Dahab & El-Goorani (1). Plates were incubated at 30 C and examined for inhibition of growth 4 days after the final streak was made.

RESULTS.—Disease severity was reduced when the tomato isolate (AT) was mixed with the tobacco isolate (VT) in all ratios tested (Table 1). Reduction in disease severity was least when equal parts of the two isolates were used, but the effect still was apparent. Results of experiments made in The Philippines and at Ithaca, N.Y., were the same, although disease development was more rapid under tropical conditions.

The two isolates cannot be distinguished from one another by their cultural characteristics; therefore, the relative growth rate of the two isolates in the inoculated plants was not determined. Neither isolate was inhibited by the other on TTC medium, but on NGCA, growth of AT was inhibited by VT when the latter was permitted to grow for 5 days before the perpendicular streak with AT was made. Growth was not inhibited, however, when the two isolates were streaked on NGCA at the same time, nor was inhibition observed on either medium when isolates were streaked against themselves under the same conditions.

Because tobacco plants were less severely diseased when both strains were introduced as mixtures, the effect of inoculating plants with the two strains independently was examined. Reax tobacco plants, 12-18 cm tall, were inoculated with aqueous suspensions (10^6 cells/ml) of either isolate, or with mixtures of the isolates as in the first experiment. The mixtures contained 10^6 cells/isolate per ml. Stems were inoculated in the upper stem in the axil of the second expanded leaf below the shoot apex, or in the lower stem in the third node above the soil line. Approximately 4-7 cm separated the two points of inoculation. Each treatment was replicated 10 times, and the plants were arranged randomly on a greenhouse bench after inoculation. Temperatures of 25-30 C prevailed in the greenhouse during the 4 weeks following inoculation, but occasional extremes of 22 or 36 C occurred for periods of 1-2 hr.

Wilt developed more slowly when tobacco plants were inoculated with the tobacco isolate (VT) at the lower stem position than when inoculated with this isolate at the axil of the second expanded leaf, but after 4 weeks the disease indices were not significantly different (Table 2). Plants that were inoculated with the tomato isolate (AT) alone did not wilt regardless of the site of inoculation. When the two isolates were mixed and introduced into the upper stem, the disease index was less than that observed when VT was introduced alone at the same concentration. However, when the mixture was introduced into the lower stem, disease severity was not reduced.

Disease severity also was reduced when isolate AT was introduced into the upper stem at the same time that VT was introduced into the lower stem (Table 2). However, when the isolates were reversed in position, no reduction of disease severity occurred.

DISCUSSION.—Disease severity diminished when mixtures of two isolates of *P. solanacearum* that differ in virulence to tobacco were introduced into tobacco stems, as compared with plants inoculated with the virulent strain alone. Presumably, this effect was an expression of host response, because tests in vitro did not indicate that the less virulent strain (AT) was antagonistic to the other (VT). The fact that wilt was less severe when isolate AT was introduced into the upper stem and VT into the lower stem also supports

TABLE 2. Symptom development in Reax tobacco plants inoculated simultaneously at one or two locations with an isolate of *Pseudomonas solanacearum* virulent to tomato and tobacco (VT) and another virulent to tomato but not to tobacco (AT)[a]

Treatment	Disease index[b]			
	7[c]	14	21	28
VT (upper stem)	0	38	42	60 a
VT (lower stem)	0	12	12	54 a
AT (upper stem)	0	0	0	2 b
AT (lower stem)	0	0	0	6 b
VT & AT (upper stem)	4	10	10	42 c
VT & AT (lower stem)	0	20	38	58 a
AT (upper stem) and VT (lower stem)	4	6	12	32 d
VT (upper stem) and AT (lower stem)	10	30	42	58 a
Check	0	0	0	4 b

[a] Ten plants/treatment inoculated with a cell concentration of 10^6 cells/ml per isolate.
[b] Disease index computed by the method of Winstead & Kelman (7). 0 = no symptoms; 20 = localized necrosis at site of inoculation; 40 = vascular browning 2 cm or more from site of inoculation; 60 = 1 or more leaves entirely wilted; 80 = all leaves wilted; 100 = plant dead. Indices having the same letter are not significantly different from one another at the .05 level, based on the t-test.
[c] Number of days after inoculation.

this conclusion. In a similar study using avirulent variants and virulent cells of *P. solanacearum* race 2 (4), Averre & Kelman (2) concluded that host response was a probable explanation for reduced disease severity. Their observations and mine indicate that avirulent variants, virulent strains of race 2, and virulent isolates of race 1 that do not induce wilt of tobacco may reduce disease severity when introduced into young tobacco stems in mixtures with strains that wilt tobacco. The results of my experiments, however, indicated that age of plant tissues may be important for expression of this phenomenon. Disease severity was not reduced when a mixture of the two isolates was introduced into older stem tissue near the soil line. Likewise, no effect on disease severity occurred when the lower stem was inoculated with the less virulent isolate (AT) alone and the upper stem with VT alone.

This study does not demonstrate that an interaction of strains of *P. solanacearum* in tobacco is the cause of uneven and relatively slow wilt development in tobacco fields of The Philippines. *Pseudomonas solanacearum* invades roots of tobacco plants in the field, and effects of strain interaction in roots were not investigated. Because strains of low virulence to tobacco are common in tobacco-producing localities of The Philippines, tobacco roots probably are invaded frequently by such strains. If this invasion stimulates a defensive mechanism in tobacco roots, the response could be an important factor in subsequent disease development in plants that are infected by a highly virulent strain.

LITERATURE CITED

1. Abo-El-Dahab, M. K., & M. A. El-Goorani. 1969. Antagonism among strains of Pseudomonas solanacearum. Phytopathology 59:1005-1007.
2. 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.
3. Breed, R. S., & J. D. Brew. 1916. Counting bacteria by means of the microscope. New York Agr. Exp. Sta. Tech. Bull. 49. 31 p.
4. Buddenhagen, I., L. Sequeira, & A. Kelman. 1962. Designation of races in Pseudomonas solanacearum. Phytopathology 52:726 (Abstr.).
5. Kelman, A. 1954. The relationship of pathogenicity of Pseudomonas solanacearum to colony appearance in a tetrazolium medium. Phytopathology 44:693-695.
6. 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.).
7. Winstead, N. N., & A. Kelman. 1952. Inoculation techniques for evaluation of resistance to Pseudomonas solanacearum. Phytopathology 42:628-634.
8. Zehr, E. I. 1969. Studies of the distribution and economic importance of Pseudomonas solanacearum E. F. Smith in certain crops in the Philippines. Philippine Agr. 53:218-223.
9. Zehr, E. I. 1971. Strains of Pseudomonas solanacearum in The Philippines as determined by cross-inoculation of hosts at different temperatures. Philippine Phytopathol. (in press).
10. Zehr, E. I. 1971. Cultural, physiological, and biochemical properties of isolates of Pseudomonas solanacearum from The Philippines. Philippine Phytopathol. (in press).