Resistance to Corynebacterium michiganense Measured in Six Lycopersicon Accessions

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The author thanks Gary V. Richardson, Biometrical Services Staff, ARS, Fort Collins, Colorado, for the statistical analysis, and Paul G. Brown and Marilyn J. Samuel for technical assistance.

Accepted for publication 15 March 1971.

ABSTRACT

Resistance to bacterial canker of tomato (*Corynebacterium michiganense*) was measured in four accessions of *Lycopersicon esculentum,* one of *L. pimpinellifolium,* and one of *L. hirsutum.* The measure of resistance was based on canker length, percentage of the stem with internal vascular discoloration, and by the number of pathogen cells per gram of stem tissue (fresh weight). Canker length correlated statistically with percentage internal discoloration, but not sufficiently to justify its use as an independent measure of resistance. Percentage internal discoloration and cells per gram of fresh tissue had an obvious positive correlation. Data tend to support the hypothesis that resistance is at least partially a matter of bacterial inhibition. Phytopathology 61:972-974.

Resistance to bacterial canker (*Corynebacterium michiganense* [E. F. Sm.] H. L. Jens.) of tomato has been reported from several sources (4, 6, 13, 14). Thyr (13) described a method for measuring resistance involving wilt symptoms. Wakimoto et al. (15) recently showed a relationship between resistance expressed as absence of wilt and reduced multiplication of the pathogen in host tissue. Strider (12) suggested that the measurement of the advance of the pathogen in host tissue (systemic infections) is a reliable measure of resistance but offered no data. He also reviewed the literature reporting possible mechanisms of pathogenesis. Subsequently, Rai & Strobel (9) reported phytotoxic glycopeptides from *C. michiganense* cultures to be more rapidly degraded by extracts from resistant than from susceptible tomato plants, therefore suggesting that resistance is expressed as the ability to degrade the toxin. However, an error in their identification of resistant and susceptible lines (13) reverses their results, and subjects their implicit hypothesis to question as to the nature of resistance to the pathogen. Reports are numerous on host-pathogen systems in which bacterial growth is retarded in resistant plants (1, 2, 10, 11).

This paper offers data showing a positive relationship between linear advance of the pathogen in host stems and cell multiplication of the pathogen. A possible explanation is offered to account for the results of Rai & Strobel (9), in view of their error in identification of resistant and susceptible accessions.

MATERIALS AND METHODS.—Six tomato accessions (seven plants each), replicated 4 times, were inoculated at the beginning of the three-leaf stage with a 4-day-old suspension of *C. michiganense.* All replications were inoculated at the same time. Three accessions of *Lycopersicon esculentum* Mill. (Highlander, Campbell 17, and Heinz 1350) are regarded as susceptible cultivars. Another *L. esculentum* accession (Bulgaria 12 [P.I. 330727]), one of *L. pimpinellifolium* (Jusl.) Mill. (Utah 20 [P.I. 344102]), and one of *L. hirsutum* Humb. and Bonpl. (P.I. 251305) were reported as exhibiting some degree of canker resistance (6, 13). Inoculation was by removal of the first true leaf at its point of attachment to the stem, and application of 5 µliters of a Ringer solution (3) suspension of the pathogen containing about 3.2×10^{11} cells/ml. Plants were grown in growth chambers with temperatures of 18 C night and 24 C day with a 12-hr photoperiod. Lighting in each chamber was 1,000 ft-c provided by high output cool-white fluorescent and regular incandescent bulbs.

Plants were grown in a sterilized mixture of peat moss, perlite, and soil (1:1:5) in 7.5-cm^2 peat pots. Soil moisture was maintained with a constant water table 6-7 cm below the soil surface (5).

Twenty-eight days after inoculation, plants were excised at ground level and the following measurements were taken for each plant: (i) plant height; (ii) length of canker at inoculation point; (iii) linear extent of vascular discoloration above and below inoculation point; and (iv) number of *C. michiganense* cells/g of stem tissue (fresh wt).

The extent of vascular discoloration was determined by removing, in cross-section, ca. 1-cm segments of stem beginning at the apex and descending until discoloration was observed. In all cases, discoloration extended below the inoculation point to ground level. The remaining stem tissue was weighed, surface disinfected in 0.5% NaOCl in 3% ethanol for 2 min, and cut into pieces about 5 mm long. The stem pieces were then aseptically pulverized in sterile Ringer solution in a mortar. The liquid suspension was decanted to remove most of the stem tissues and was passed through nine 10-fold dilutions. Five of the dilutions from each sample were plated on nutrient agar in triplicate.

Because it was expected that some lines would have many more cells/g of tissue than others, the same dilutions were not plated for all lines. After the first replicate was plated and cell counts were apparent, adjustments were made so that the most appropriate dilutions were plated for each accession in the remaining replicates. Heterogeneity of the pulverized tissues, however, precluded highly accurate cell counts.

RESULTS AND DISCUSSION.—Resistance to bacterial

TABLE 1. Reaction of six *Lycopersicon* accessions, including three species, to *Corynebacterium michiganense* as measured by canker length, percentage of plant height with internal discoloration, and pathogen cells per g of fresh tissue[a]

Accession	Canker length	Vascular discoloration[b]	Pathogen cells/g fresh tissue
	mm	%	$\times 10^5$
Lycopersicon esculentum			
Highlander	13.8 a	98.0 a	1,500,398
Campbell 17	10.2 ab	71.2 b	256,888
Heinz 1350	9.3 b	64.0 b	29,908
Bulgaria 12 (P.I. 330727)	6.7 bc	32.9 c	153
L. pimpinellifolium			
Utah 20 (P.I. 344102)	2.5 c	8.5 d	50
L. hirsutum			
P.I. 251305	3.5 c	6.8 d	8

[a] Data represent means of four replicates of seven plants each. Figures with the same letter do not differ significantly (.05) by Tukey's mean separation test.

[b] Portion of main stem with visible vascular discoloration.

canker in tomato ranged from essentially none in the cultivar Highlander to a high level in P.I. 251305 and Utah 20 (P.I. 344102). Restriction of vascular discoloration, expressed as percentage of stem length with discoloration, was a good measure of resistance to the canker pathogen, thus confirming Strider's statement to this effect (12). A positive correlation was apparent between percentage internal discoloration and pathogen cells/g of fresh tissue (Table 1), although a statistical correlation was not attempted due to the very large interaccession differences in cell counts. A positive correlation was found between canker length and percentage internal discoloration. However, it was not sufficient to justify using canker length as an independent measure of resistance to *C. michiganense*.

The reversal in the results of Rai & Strobel (9) brought about by their incorrect identification of resistant and susceptible accessions, means that the phytotoxic glycopeptide causes more rapid wilting in the resistant Utah 20 than in the susceptible line P.I. 283907 (13). Also, according to their results, the glycopeptide is more slowly degraded in the resistant than in the susceptible line. Assuming that the only error by Rai & Strobel was in plant identification, the following interpretation may explain their results in view of this unexpected reversal.

Wilting, caused by an internal water deficit, is known to result in stomatal closure which in turn reduces transpirational water loss (7, p. 86). A reasonable response for a plant with resistance to a given pathogen might be to more slowly degrade a toxin which is indirectly acting to reduce water loss in the plant. Following the same logic, one might expect a susceptible plant to rapidly degrade a toxin which might be capable of reducing water loss. If such is the case, the toxin may or may not play an important role in disease expression. However, a recent report (8) lends some support to the theory expressed above. That work shows a hypersensitive response in resistant excised apple leaves to *Venturia inaequalis* (Cke.) Wint., expressed as more rapid wilting than in suscepts.

One might expect a toxin, which causes wilting as readily as Rai & Strobel (9) indicate, to be active in areas where the pathogen is active. Wilting is nearly always restricted to areas of the plant where vascular discoloration is evident. Although the bacteria are found in much greater numbers in such areas, the pathogen can also be found in substantial quantities in areas of the stem unaffected by vascular discoloration and above the highest point at which leaves are wilted.

However, it is my firm belief that canker-resistant tomato accessions express their resistance, not so much by their action or inaction upon a phytotoxic glycopeptide, but by action directly upon the bacteria. Support of this theory is evident (Table 1) where resistant accessions show greatly reduced numbers of the pathogenic bacteria over susceptible accessions. Attempts to reveal an inhibitor in extracts from inoculated tomatoes, both resistant and susceptible, were unsuccessful. However, it is possible that any inhibitor present was destroyed during extraction procedures. These efforts are being continued.

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