Virulence of Corynebacterium michiganense Isolates on Lycopersicon Accessions

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ABSTRACT

The virulence of seven isolates of *Corynebacterium michiganense*, collected from six geographical areas of the United States, was measured on five accessions of *Lycopersicon esculentum*, one of *L. pimpinellifolium*, and one of *L. hirsutum*. Data on stunting of host plants and on vascular discoloration were subjected to analysis of variance which showed significant differences among isolates and accessions. Highly significant interaction occurred when all three species of *Lycopersicon* were tested; but only borderline interaction occurred with internal discoloration, and none with stunting, when only *L. esculentum* accessions were subjected to analysis. Evidence supports the conclusion that resistance to bacterial canker in the *Lycopersicon* accessions tested is horizontal, and that isolates differ in degrees of aggressiveness. To obtain adequate levels of resistance in tomato lines, a backcross breeding program is suggested. Also, highly aggressive pathotypes should be employed in a breeding program to maintain an acceptable level of resistance.

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Variations in the virulence of isolates of *Corynebacterium michiganense* (E. F. Sm.) H. L. Jens., the cause of the bacterial canker in tomato, have been reported on several occasions and were reviewed recently (5). Baines (1) reported what may have been differential virulence when he isolated from *Solanum douglasii*, a bacterium similar to *C. michiganense* that reinfected *S. douglasii* as well as tomato. However, a tomato isolate failed to infect *S. douglasii*.

More recently, variations in virulence of *C. michiganense* were shown to occur on the tomato

cultivar Manapal (6). The authors indicated that a special inoculation technique was required to reveal the differences.

The reports cited above, and different levels of disease severity observed in field plantings from year to year and in different geographical locations within a single year, suggested the occurrence of different pathotypes in the pathogen population. An investigation of tomato isolates of the pathogen from six different geographical regions was undertaken to determine their virulence on several *Lycopersicon* accessions exhibiting different levels of resistance to a single isolate.

MATERIALS AND METHODS.—Plants of seven *Lycopersicon* accessions were grown and tested for their reactions to seven isolates of *C. michiganense*. Plants were grown individually in 3-inch peat pots containing a mixture of peat, perlite, and soil (1:1:5). Accessions tested were *L. esculentum* Mill. unless otherwise stated: 1) Utah 20 (P.I. 344102), *L. pimpinellifolium* (Jusl.) Mill.; 2) P.I. 251305, *L. hirsutum* Humb. and Bonpl.; 3) Bulgaria 12 (P.I. 330727); 4) Homestead; 5) Heinz 1350; 6) Highlander; and 7) Campbell 17. Accessions 1, 2, and 3 have exhibited degrees of resistance to *C. michiganense* (3, 7). Bacterial isolates used in this study were as follows: A) 829-N, North Carolina tomato, D. L. Strider, North Carolina State University, Fletcher; B) Cm 3, Colorado tomato stem, author's collection; C) Cm 4, Nebraska tomato, M. L. Schuster, University of Nebraska, Lincoln; D) 829-S, see isolate A; E) 69-2, Florida tomato, R. E. Stall, University of Florida, Gainesville; F) Cm 7, Ohio tomato fruit spot, author's collection; G) Cm 15, Indiana tomato fruit spot, W. C. Virgin, Del Monte Corporation, P.O. Box 36, San Leandro, Calif.

Four-day-old cultures grown on nutrient agar and suspended in Ringer solution (2) provided the inoculum. Inoculum density was about 10^8 cells/ml and was determined by optical density, which was correlated with viable counts. Plants were inoculated at the beginning of the 3-leaf stage (4 weeks old) by the excising of the first true leaf at its point of attachment and application of 5 μliters of the appropriate inoculum. Seven plants were inoculated for each accession-isolate combination, except that one of these plants was inoculated with sterile Ringer solution as a control to learn the extent of cross-contamination between isolates and degree of stunting by the pathogen. This allowed seven noninoculated control plants of each accession. The experiment was arranged in a randomized block design with three replications in a greenhouse where temperature ranged from an average 14 C low at night to 34 C high during the day. Photoperiod (sunrise to sunset) averaged about 13.5 hr for the period of 11 weeks from planting until termination of the experiment.

At termination, plants were excised at the point of inoculation. I measured height of excised plants, and determined the extent of internal vascular discoloration by cutting stems transversely about every centimeter and observing them for the vertical extent of discoloration. The percentage of stem length with internal vascular discoloration was computed (8). Percentage reduction in plant height (stunting) was determined for all treatments. Both criteria were used in comparing the resistance of accessions and the virulence of the isolates. Analysis of variance for vascular discoloration and stunting was computed, and Tukey's mean separation (.05) was determined for accessions within isolates and for isolates within accessions. Analysis of variance was also computed for vascular discoloration and stunting of the five *L. esculentum* accessions with all seven isolates.

RESULTS.—Data on vascular discoloration and stunting showed significant differences among accessions within isolates (Fig. 1), and among isolates within accessions (Fig. 2). However, several of the accessions reacted to isolates similarly, as did some of the isolates to accessions.

Analysis of variance for all accessions revealed a highly significant interaction with vascular discoloration and stunting; but when only *L. esculentum* accessions were subjected to analysis, no interaction was found with stunting and only marginal interaction with vascular discoloration.

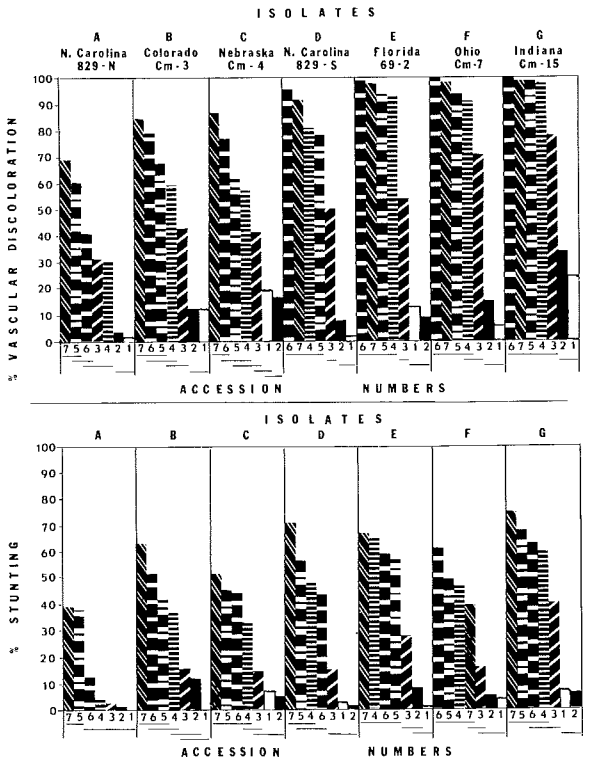

Fig. 1. *Lycopersicon* accessions ranked by internal vascular discoloration and by stunting from *Corynebacterium michiganense* isolates. Data represent means of three replications. Accessions joined by a common line under a given isolate do not differ significantly (.05) by Tukey's w procedure. Mean separation values (Tukey's HSD) are 22.7 for vascular discoloration and 25.0 for stunting.

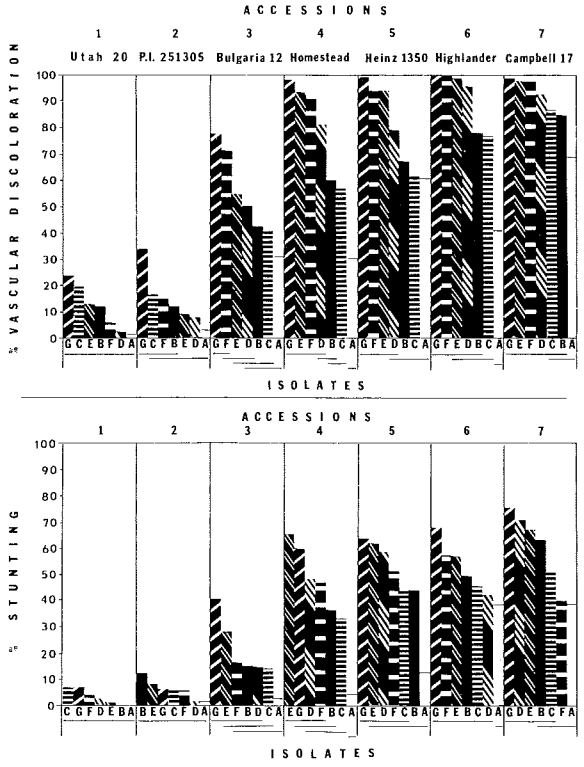

Fig. 2. *Corynebacterium michiganense* isolates ranked by internal vascular discoloration and by stunting of *Lycopersicon* accessions. Data represent means of three replications. Isolates joined by a common line under a given accession do not differ significantly (.05) by Tukey's w procedure. Mean separation values (Tukey's HSD) are 22.7 for vascular discoloration and 25.0 for stunting.

Disease severity ranged from high to low. Accessions 1 and 2, *L. pimpinellifolium* and *L. hirsutum*, respectively, always had the lowest level of disease and never differed significantly from each other regardless of the isolate or means of measurement used (Fig. 1). Accessions 4, 5, 6, and 7 had the highest disease levels, particularly when inoculated with isolates E, F, and G. Accession 3 was intermediate. Among the seven isolates, A caused the lowest level of disease, whereas isolates D, E, F, and G caused the most, especially on accessions 4, 5, 6, and 7. Isolates B and C were intermediate, but usually not significantly different from both the most and least virulent (Fig. 2).

DISCUSSION.—There are no significant differences in the way accessions or isolates are ranked by vascular discoloration and by stunting. Stunting, for the most part, is directly correlated with vascular discoloration. It appears as an effect of multiplication and advance of the pathogen in host tissues (measured perhaps inadequately by vascular discoloration), and in all cases it had lower or equal mean values for a given accession-isolate combination than did vascular discoloration. Vascular discoloration accounts only for the advance of the pathogen up the stem, and although it says nothing directly of the actual multiplication of the pathogen, previous work has shown them to be positively correlated (8). Since the extent of vascular discoloration may not always be clearly visible, measurement of it may be a greater source of experimental error than measurement of stunting. However, because the lag between vascular discoloration and stunting is not the same for all accession-isolate combinations, the author tends to favor vascular discoloration over stunting as a means of differentiating among pathotypes and levels of host resistance.

It appears from data on stunting that all accessions have at least a low level of resistance, regardless of the isolate used, since the largest mean reduction in plant height is approximately 75%. On the other hand, accessions 4, 5, 6, and 7 fail to show significant levels of resistance to isolates D, E, F, and G as determined by vascular discoloration. The isolates appear to fall into three fairly distinct levels of aggressiveness (4). Isolate A is the least aggressive; isolates B and C next and intermediate in strength; and isolates D, E, F, and G are grouped together as most aggressive.

Highly aggressive pathotypes should be used not only in selecting breeding lines for high level resistance, but also in rigorous testing programs designed to maintain resistance in resistant cultivars following their development.

The levels of canker resistance evident in Fig. 1 indicate that inheritance is polygenic (9). A backcross program appears adequate for breeding canker resistance; although composite crosses as suggested by van der Plank (9) may also prove successful.

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