Relation of Age of Plants, Temperature, and Inoculum Concentration to Bacterial Canker Development in Resistant and Susceptible Lycopersicon spp.

R. L. Forster and E. Echandi

Graduate Fellow and Professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27607.

Journal Series Paper No. 3898 of the North Carolina State University Experiment Station, Raleigh.

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named, nor criticism of similar ones not mentioned.

Research supported in part by National Science Foundation Grant No. GB 28951 and National Defense Education Act Title IV Fellowship.

The authors thank H. J. Kirk, R. J. Downs, and the staff of the Southeastern Plant Environment Laboratory, Raleigh, for assistance in conducting this research.

Accepted for publication 3 January 1973.

ABSTRACT

Plants of bacterial canker-resistant *Lycopersicon hirsutum* (P.I. 251305), *L. esculentum* (P.I. 340905), and *L. esculentum* × *L. pimpinellifolium* ('MR 4' and 'Bulgaria 12') and of susceptible *L. esculentum* ('Manapal') and *L. peruvianum* (P.I. 251306) were grown from seed in a phytotron at 26/18 C day/night temperature superimposed on a photoperiod of 9 hr of high-intensity light plus 1 hr of incandescent light. Three days before inoculation, plants were transferred to the different temperature regimes. Plants were inoculated by stabbing the stem above the cotyledonary leaves with a dental root-canal file dipped in a suspension containing 10^7 cells/ml of *Corynebacterium michiganense*. "Resistance" in this paper refers to the resistance to infection when the pathogen is introduced directly into the vascular system. Differences in disease ratings between resistant and susceptible accessions were greater in 5-week-old plants (16- to 24-cm tall) than in 4-, 6-, and 7-week-old plants. The age × accession interaction was significant. Five-week-old plants at 24/18 C showed greater differences in disease ratings than those at 20/18, 28/18, or 32/18 C, and the temperature × accession interaction was significant. Inoculum that contained 10^9 cells/ml induced larger differences in disease ratings in 5-week-old plants at 24/18 C than inoculum with 10^5 and 10^7 cells/ml, but inoculum with 10^7 cells/ml induced differences almost as great as that with 10^9 cells/ml.

Phytopathology 63:773-777

Additional key words: screening for resistance.

Resistance to bacterial canker [*Corynebacterium michiganense* (E. F. Sm.) H. L. Jens.] of tomato has been reported from several sources (4, 7, 9, 11, 12) but a screening procedure considering both plant and environmental conditions has not been developed. Hassan et al. (4) screened solanaceous species for resistance by spray-inoculating cotyledons with an aqueous suspension of *C. michiganense*. Thyr (9) inoculated tomato seedlings at the beginning of the three-leaf stage by excising the first true leaf at its point of attachment and applying inoculum to the wound. Eight weeks after inoculation, plants with one or more unwilted shoots were considered resistant, whereas those with all shoots dead or at least partially wilted were considered susceptible. Strider (8) compared root and stem inoculations of 14- and 28-day-old tomato plants. Disease development was most rapid and uniform in 14-day-old plants that had been stem-inoculated, and root inoculation methods were judged undesirable due to nonuniform results.

Kendrick & Walker (5) studied the effect of plant age in relation to bacterial canker development and concluded that the disease progressed at about the same rate in plants of each age group. However, symptoms appeared later in the older plants. Blood (1) indicated that 28 C was optimal for disease development for both soil and air. Thyr (10) determined the percentage of infection in 'Highlander' tomato seedlings inoculated with varying concentrations of inoculum and reported that tomato seedlings become infected with as few as five cells per plant when the inoculum was introduced directly into the xylem. Ercolani (2) reported that the aetiology of response of tomato to *C. michiganense* is best described by the hypothesis of independent action. He indicated that this hypothesis is conjectural in nature, and the possibility is provided for the plant response to be produced by the progeny of only one of the inoculated bacteria. An abstract of the present paper has been published (3).

The objectives of this study were: (i) to determine the effects of plant age, temperature during the infection period, and inoculum concentration upon infection of resistant and susceptible tomato accessions; and (ii) to develop a screening procedure using the optimum for each factor based on the largest differences between resistant and susceptible accessions. As used in this paper, the term "resistance" refers to the resistance to infection exhibited after the pathogen had been introduced directly into the vascular system of the host.

MATERIALS AND METHODS.—Plants of bacterial canker-resistant *Lycopersicon hirsutum* Humb. & Bonpl. (P.I. 251305), *L. esculentum* Mill. (P.I. 340905), and *L. esculentum* × *L.*

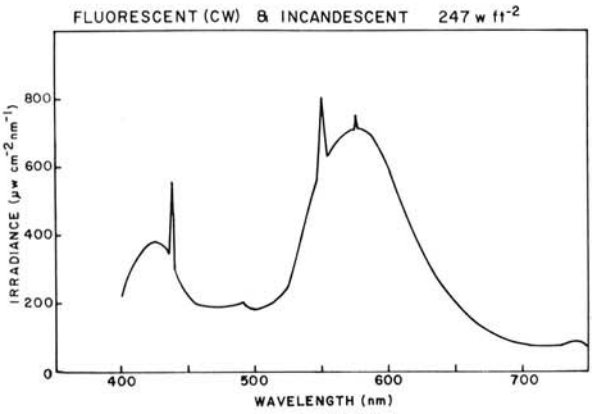

Fig. 1. Spectral energy distribution in controlled environment room at top of pot in which tomatoes were grown. Light derived from cool-white (CW) fluorescent and incandescent sources. Total irradiance (area under the curve) equals 43,000-48,400 lx (4,000-4,500 ft-c).

pimpinellifolium ['MR 4' (E. Echandi, *unpublished*) and 'Bulgaria 12'] and of susceptible *L. esculentum* ('Manapal') and *L. peruvianum* Mill. (P.I. 251306) were grown from seed in Jiffy Mix® (W. R. Grace Co., Travelers' Rest, S. C.) in controlled environment rooms (CER's) in the North Carolina State University phytotron. Day/night air temperatures of 26/18 ± 1/4 C superimposed on a photoperiod of 9 hr of high-intensity light (43,000-48,400 lx = 4,000-4,500 ft-c) plus an additional hour of incandescent light (3,200 lx = 300 ft-c) before the start of day temperature conditions. Air temperature was measured with No. 22-guage thermocouples. Figure 1 shows the spectral energy distribution (measured with an ISCO spectroradiometer) of the high-intensity light derived from 4.8 kW incandescent and 18 kW fluorescent sources. Light intensity was measured with a cosine-corrected Gamma Scientific photometer. CO_2 concentration in the CER ranged between 300-400 ppm. The downward air flow had an average velocity of 30.5 m/min. All plants received a standard phytotron nutrient solution (6) 5 days a week and deionized water on weekends. Seedlings were transplanted after 12 days into sterilized coarse sand in 10-cm diam plastic pots.

Three days before inoculation, CER temperature regimes were programmed to the requirements of the experiments described below. Treatments were randomized according to a split-plot design in which the main treatment (either plant age, temperature, or inoculum concentration) was randomly assigned to a CER and the subtreatments (accessions) were randomly assigned within each main treatment.

A virulent isolate of *C. michiganense* (CM7A) obtained from diseased plants in western North Carolina was maintained on nutrient agar at 4 C and used as a stock culture. Plants were inoculated by stabbing the stem twice above the cotyledonary leaves with a dental root-canal file (Style D, No. 1, Kerr Manufacturing Co., Detroit, Mich.) previously dipped in a suspension of *C. michiganense* cells grown for 3 days in nutrient broth shake culture at 25 C. Cultures at this stage were in the mid-log, to early transition, phase of growth. A file dipped in a 10^7 cells/ml suspension of *C. michiganense* carried approximately 5,700 cells. Therefore, an inoculated plant received approximately 1.1 × 10^4 cells. Inoculum was standardized with a Spectronic 20 colorimeter. Noninoculated control plants of each accession were maintained in all experiments.

Disease ratings of wilt were made on each plant when the sand was near field capacity and were based on the following subjective scale: 0 = no wilting; 1 = one lower leaf wilted; 2 = two to four lower leaves or fewer than half the leaves wilted; 3 = half to three-quarters of the leaves wilted; 4 = two of the top five leaves or more than three-quarters of the leaves wilted but the terminal leaves of the main shoot not wilted; and 5 = terminal leaves of main shoot and most leaves wilted or dead.

RESULTS.—*Effect of plant age.*—Seeds were sown at 1-week intervals to obtain plants 4, 5, 6, and 7 weeks old at the time of inoculation. Plant heights are shown in Table 1. Air temperature was maintained at 26/18 C throughout the experiment.

Wilt symptoms first appeared on lower leaves of 4-week-old Manapal and *L. peruvianum* plants 9 days after inoculation. Sixteen days after inoculation, plants in all accessions showed wilt symptoms. Large, open cankers (20 to 30-mm long) were visible at the inoculation sites in *L. peruvianum* and *L. hirsutum* plants but their presence or length was not correlated with foliage wilt. Six- and 7-week-old plants of Bulgaria 12 and P.I. 340905 exhibited initial wilt 3-4 weeks after inoculation, at which time Manapal and *L. peruvianum* plants of similar age had advanced wilt symptoms.

Differences in disease ratings between resistant and susceptible accessions were greatest in plants inoculated at the 5-week-old stage (Table 2). These differences were computed for each age by summing the differences between the disease rating for Manapal and the disease rating for each of the resistant accessions. These differences were significant at the 0.05 level. A significant age × accession interaction was detected, which indicated that the effect of accession depended on plant age. Except for *L. hirsutum*, plants which were older at the time of

TABLE 1. Relationship of age to plant height among selected tomato genotypes grown in a phytotron

Accession	Plant age (wk)			
	4	5	6	7
	Height (cm)			
Lycopersicon hirsutum	3	16	31	45
Bulgaria 12	9	22	41	48
P.I. 340905	6	21	43	63
MR 4	11	22	38	55
Manapal	10	24	40	56
L. peruvianum	8	19	35	66

TABLE 2. Effect of age, daily air temperature and inoculum concentration on severity of bacterial canker in tomato accessions[a]

Level of experimental factor	Accession					
	Lycopersicon hirsutum	Bulgaria 12	P.I. 340905	MR 4	Manapal	*Lycopersicon peruvianum*
Age (weeks) when inoculated using 10^7 cells/ml. Plants were maintained at 26-C day and 18-C night temperatures						
4	0.4	2.5	1.5	- [b]	3.2	4.5
5	1.8	1.6	0.2	-	3.6	2.9
6	3.2	1.4	0.6	-	3.4	3.2
7	2.0	1.9	0.5	-	2.4	1.5
For accessions within age, LSD (.05) = 1.8						
Daily air temperature (C) (15-hr nights had constant 18 C). Plants were 5 weeks old when inoculated using 10^7 cells/ml						
20	0.2	2.4	0.8	1.8	3.1	3.4
24	0.8	2.1	1.0	1.1	4.0	3.5
28	2.8	2.0	2.9	1.1	4.8	4.2
32	1.1	2.2	2.1	0.6	4.0	4.0
For accessions within temperature, LSD (.05) = 1.2						
Inoculum concentration (cells/ml). Five-week-old plants were maintained at 24-C day and 18-C night temperatures						
10^3	0	0.1	0	0	0.5	1.1
10^5	2.8	1.4	1.5	1.1	2.9	3.6
10^7	2.1	2.8	2.8	2.0	4.0	3.6
10^9	3.8	3.1	2.9	2.9	4.9	3.9
For accessions within concentration, LSD (.05) = 1.3						

[a] 0 = no wilting; 5 = entire plant wilted (see text). Numbers in table are means of individual disease ratings of eight plants/treatment taken 30 days after inoculation.

[b] Ratings eliminated for statistical purposes due to many missing plots.

inoculation had lower disease ratings than plants which were younger.

In some plants of *L. hirsutum*, the main stem above the inoculation point wilted and died 20-25 days after inoculation. Lateral shoots developed at the base of the stem and were free of wilt symptoms through the remainder of the experiment. A similar response occurred in some plants of *L. peruvianum* 14-16 days after inoculation.

During this experiment, MR 4 and *L. hirsutum* developed intumescences on leaves, petioles, and stems. This disorder caused abscission of lower leaves of 6- and 7-week-old plants of MR 4. For statistical purposes, all ages of MR 4 were deleted from the analysis of variance in this experiment.

Effect of temperature.—Three days before inoculation, 5-week-old plants were transferred to 20/18, 24/18, 28/18, and 32/18 C temperature regimes for acclimatization. Plant heights at the time of inoculation compared closely with heights of 5-week-old plants in the age experiment.

As in the age experiment, Manapal and *L. peruvianum* were first to exhibit wilt. Plants were most susceptible when grown at 28/18 C (Table 2). However, plants at 24/18 C showed greater differences in disease ratings between resistant and susceptible accessions than those at 20/18, 28/18, and 32/18 C. Effects of accession and temperature on disease development were both highly significant ($a = 0.01$). Significance of the temperature × accession interaction was primarily due to the resistant response of MR 4 and Bulgaria 12. Disease ratings were generally higher for plants grown in the warmer temperature regimes and lower for plants grown in the cooler regimes. MR 4 plants grown under warmer conditions, however, had lower disease ratings than those grown under cooler conditions. Temperature during the infection period apparently had no effect on disease development in plants of Bulgaria 12.

Effect of inoculum concentration.—Three days before inoculation, 5-week-old plants were transferred to 24/18 C CER's where they remained throughout the experiment. Bacterial suspensions containing 10^9, 10^7, 10^5, 10^3 cells/ml were prepared in sterile distilled water from nutrient broth shake cultures of *C. michiganense*. Inoculations were completed within 15 min of initial dilution.

Wilt first appeared on plants of *L. peruvianum* and Manapal inoculated with 10^9 cells/ml. Susceptible plants inoculated with 10^5 cells/ml developed symptoms at the same time as resistant plants inoculated with 10^7 and 10^9 cells/ml. Most plants inoculated with the lowest concentration showed no wilt and no external necrosis of tissue around the inoculation site and only slight darkening in the vascular tissue and pith near the inoculation site.

Within a particular level of inoculum, most accessions exhibited a wide variation in individual plant responses, especially when observed 2 weeks after inoculation. However, Bulgaria 12, MR 4, and Manapal showed less variation than the other accessions. Disease ratings increased with time, and resistance of MR 4 plants persisted longer than in any other accession.

Except for *L. peruvianum*, higher inoculum concentrations resulted in higher individual disease ratings (Table 2). *L. peruvianum* was not affected by an increase from 10^5 to 10^9 cells/ml. Plants inoculated with 10^9 cells/ml had greater differences in disease ratings between resistant and susceptible accessions than those inoculated with 10^5 or 10^7 cells/ml, but 10^7 cells/ml produced differences almost as great. The effect of inoculum concentration on disease development was highly significant ($a = 0.01$).

DISCUSSION.—Qualification of the term "resistance" is necessitated by the different responses of P.I. 340905, MR 4, and Bulgaria 12 to foliar infection and to stem puncture inoculation. These accessions were not resistant to foliar blight when subjected to foliar inoculation.

The results show large differences between the high and low levels of all three factors tested. Under the conditions of these experiments, all three factors were of similar importance. Temperature × age × inoculum concentration experiments in a factorial analysis should indicate which of the three factors is most important in the development of bacterial canker. Large differences between resistant and susceptible accessions may be detectable in young plants inoculated with low concentrations of bacteria, but smaller differences would go undetected. Observations in the CER and greenhouse seemed to indicate that plant age may not be as important as plant height in resistance to bacterial canker. The results of the temperature experiment confirm those of Blood (1) and indicate that slightly less than optimal temperature for bacterial growth is necessary for maximum separation of levels of resistance. Photoperiod variation might alter differences in resistance observed in any or all of the experiments reported in this paper.

Plants inoculated with 10^9 cells/ml had the greatest differences in disease ratings between resistant and susceptible accessions. However, in testing for resistance, inoculations with 10^7 cells/ml is suggested. Differences between resistant and susceptible accessions, inoculated with these two concentrations, were similar. In a preliminary experiment 10^7 cells/ml produced greater differences than the higher concentration. Also, *L. hirsutum* had an unusually high disease rating following inoculation with 10^9 cells/ml; therefore, this concentration might eliminate valuable sources of resistance.

The response of *L. peruvianum* to inoculum concentration was unusual, because the disease ratings were essentially the same over a 10^4 factor difference in concentration. This indicates a high susceptibility of the host. It would have been necessary to make more frequent disease ratings for *L. peruvianum* soon after inoculation to distinguish differences due to different inoculum concentrations.

Results obtained with the lowest inoculum concentration confirm Thyr's work (10) in which he found that infection occurred using as few as five cells/plant. In our study we computed the number of cells/plant inoculated with the lowest inoculum level to be between one and two. The difference might be due to different isolate virulences or different resistances of the hosts.

The results in this study confirm the previously reported resistance in Bulgaria 12, P.I. 340905, and *L. hirsutum* (4, 9, 11). The resistance of inoculated plants of accessions under CER conditions is similar to that expressed by the same accessions inoculated by stem puncture and grown under greenhouse or field conditions (*unpublished data*).

Wilt symptoms also develop similarly in phytotron and greenhouse studies (*unpublished data*). Unilateral wilting of leaflets preceded wilt of the entire leaf. However, greenhouse-inoculated plants exhibited more mushy, discolored pith at the inoculation site. Our data indicate that resistant and susceptible accessions can be separated by inoculating 5-week-old plants by stem puncture using a dental root canal file dipped in a 10^7 cells/ml suspension of *C. michiganense* and incubating those plants at 24/18 C day/night temperatures.

LITERATURE CITED

1. BLOOD, H. L. 1930. Bacterial canker of tomato. Ph.D. Thesis. Univ. Wisc., Madison.
2. ERCOLANI, G. L. 1967. Bacterial canker of tomato. II. Interpretation of the aetiology of the quantal response of tomato to Corynebacterium michiganense (E. F. Sm.) Jens. by the hypothesis of independent action. Phytopathol. Mediterr. 6:30-40.
3. FORSTER, R. L., & E. ECHANDI. 1972. Effect of age, temperature, and inoculum concentration on bacterial canker development in resistant and susceptible Lycopersicon spp. Phytopathology 62:757 (Abstr.).
4. HASSAN, A. A., D. L. STRIDER, & T. R. KONSLER. 1968. Application of cotyledonary symptoms in screening for resistance to tomato bacterial canker and in host range studies. Phytopathology 58:233-239.
5. KENDRICK, J. B., JR., & J. C. WALKER. 1948. Predisposition of tomato to bacterial canker. J. Agr. Res. 77:169-186.
6. RAPER, C. D., JR., & W. H. JOHNSON. 1971. Factors affecting the development of flue-cured tobacco grown in artificial environments. II. Residual effects of light duration, temperature, and nutrition during growth on curing characteristics and leaf properties. Tob. Sci. 15:76-80.
7. STRIDER, D. L. 1969. Bacterial canker of tomato caused by Corynebacterium michiganense – A literature review and bibliography. N. C. Agr. Exp. Stn. Tech. Bull. 193. 110 p.
8. STRIDER, D. L. 1970. Tomato seedling inoculations with Corynebacterium michiganense. Plant Dis. Reptr. 54:36-39.
9. THYR, B. D. 1968. Resistance to bacterial canker in tomato, and its evaluation. Phytopathology 58:279-281.

10. THYR, B. D. 1968. Bacterial canker of tomato: inoculum level needed for infection. Plant Dis. Reptr. 52:741-743.
11. THYR, B. D. 1969. Additional sources of resistance to bacterial canker of tomato (Corynebacterium michiganense). Plant Dis. Reptr. 53:234-237.
12. THYR, B. D. 1971. Resistance to Corynebacterium michiganense measured in six Lycopersicon accessions. Phytopathology 61:972-974.