Inheritance of Tolerance to Erwinia carotovora in Florida MH-1 Tomato

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ABSTRACT

Tolerance of fruit from certain tomato lines to bacterial soft rot was found to be tolerance to *Erwinia carotovora*. The tolerance to *E. carotovora* reported for cultivar Florida MH-1 (MH-1) came from the Heinz-3 (H-3) parent. The fruit from an F_1 of a Walter × MH-1 cross were as tolerant as those from the tolerant parent, MH-1. Fruit from the F_1, F_2, P_1 and P_2 of a cross between the tolerant lines MH-1 and H-3 were equally tolerant by a short-term decay method of evaluation. However, differences existed when a new rating system, index of survival, was used. H-3 was more tolerant than MH-1 or the F_2 and the F_1 was more tolerant than the F_2. Index of survival values were applied to individual F_2 plants from the MH-1 × H-3 cross; and, in another season, to plants of the F_2 generation from H-3 × Walter, Walter × H-3, H-3 × MH-1, and MH-1 × Walter crosses. The index of survival values from the F_2 plants from the reciprocal cross H-3 × Walter and Walter × H-3 were tested for homogeneity of distribution and were not significantly different, $P = 0.10$. The values for the F_2 plants from the latter cross and the MH-1 × Walter crosses were distributed normally ($P = 0.75$-0.90) while those from the combined H-3 × MH-1 and MH-1 × H-3 and the MH-1 × H-3 from the first season did not seem to fit a normal distribution, $P = 0.25$ and 0.10, respectively. Tolerance to *E. carotovora* in inoculated tomato fruit is a polygenically controlled characteristic in which dominance is complete or nearly so.

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Additional key words: bacterial populations, *Lycopersicon esculentum* breeding for disease resistance, bacterial soft rot, postharvest decay.

Tolerance to *Erwinia carotovora* (L. R. Jones) Holland in fresh market tomato fruit was described as a delay in the appearance of visible lesions at the sites of inoculated wounds (1). Fruit from Florida MH-1 (MH-1) and Homestead-24 tomato plants were more tolerant of *E. carotovora* than were those from Walter. The susceptibility of Walter probably accounted for extensive losses to bacterial soft rot in some of the first commercial shipments of that cultivar.

Florida MH-1, one of the more tolerant cultivars of those tested in the earlier reports (1, 2) was originally selected from an F_2 population derived from the cross Walter × Heinz-3 (H-3). Heinz-3 had several desirable characters, and, apparently, some resistance to preharvest fruit decays. In this paper, we report results of tests on the tolerance of H-3 to *E. carotovora,* and on the inheritance of that tolerance.

MATERIALS AND METHODS.—Fruit were hand-harvested from tomato plants that had been grown with full-bed paper or plastic mulch by the method of Geraldson (5). Fruit from individual treatments were bulked, divided into 10-fruit replicates, and arranged on plastic trays so that three treatments were represented on each tray. The tolerance of the fruit from individual plants was assessed from 10 fruit that were harvested from each plant (each fruit was handled separately).

Fruit were inoculated by pins dipped into bacterial suspensions prepared from 24-hour nutrient broth cultures of *E. carotovora* (1, 2). Inoculated fruit were stored at 21 C and approximately 95% relative humidity, and were examined every 1-2 days for soft-rot lesions.

The rate of expansion of soft rot lesions in tomato fruit was measured on fruit stored for 24 hours after the appearance of lesions. The rate of soft rot development was expressed as the percentage of the fruit that had rotted by 24 hours after a visible lesion had first appeared.

Populations of soft-rotting bacteria in wounds in fruit of Walter, H-3, and MH-1 were determined by dilution-plate analyses at various intervals after inoculation. A wounded area (four needle holes) was removed immediately after inoculation from each of five different fruit from each cultivar and was crushed in half-strength buffered saline (pH = 7.0) (1) with a glass stirring rod. Additional wounds were sampled at 18, 25, 48, and 76 hours. Ten-fold dilutions were made when required and 0.2 ml volumes were plated on a crystal violet polypectate medium prepared by the method of Cupples and Kelman (4). Depressions in the medium were counted 24-48 hours after plating.

Decay was expressed as the percentage of fruit with macroscopic lesions at a given interval after inoculation. For longer storage periods, however, two alternative methods of rating decay were used. In the first, called the "adjusted decay method," all fruit that had been ripe for at least three days before visible lesions appeared were considered healthy. The second method, called the "index of survival method," was used only when fruit were stored until almost all had lesions. In this system, green fruit with lesions were given a rating of 1, pink fruit a rating of 4, and ripe fruit were rated 7 plus the number of days that the fruit had been ripe. Ripe fruit were those with no green and little or no pink coloration. The term "ripen" was used loosely to denote the development of a red coloration.

Means of different treatments were compared by the Duncan's new multiple range test after an analysis of variance had indicated a significant F value. All percentage data were transformed by the arcsin transformation.

The rate of lesion expansion was analyzed by Student's *t*-test. Analysis of the F_2 population for inheritance of tolerance to *E. carotovora* was based on the chi-square

test for goodness-of-fit of the "index-of-survival" values for fruit from individual F_2 plants to a normal curve.

RESULTS.—Fruit from H-3, MH-1, and Homestead-24 all had significantly less soft rot than those from Walter during two different growing seasons (Table 1). During the first season enough fruit were available for a test using a 10-fold greater bacterial suspension. The results were the same as those from the tests in which the lower population was used.

Fruit from Homestead-24 did not ripen as rapidly as those of the other lines. If ripening was taken into account and the tolerances were compared by the adjusted decay method at 15 days (Table 2), H-3 was the most tolerant and MH-1 was next most tolerant. Homestead-24 was significantly more susceptible than either MH-1 or H-3.

Fruit from Walter, MH-1, and H-3 were tested for factors which might be inhibitory to *E. carotovora*. The average population of soft-rotting bacteria was significantly higher in wounds in Walter tomato fruit than in MH-1 or H-3 fruit at 18 hours after inoculation (Table 3). Similar differences existed at 25 and 48 hours; however, significance could not be attributed to those differences because of up to 10-fold differences in the bacterial populations in wounds in tomatoes of the same line. By 76 hours after inoculation, the populations of soft-rotting bacteria in wounds were similar.

The rate of lesion enlargement in fruit from a susceptible and a tolerant line were studied to determine if tolerance also involved inhibition in the rate of soft-rotting once a lesion had developed. Lesions in the more tolerant fruit developed as fast if not faster than those in the more susceptible fruit.

The possibility that tolerance to *E. carotovora* was an inherited characteristic was suggested from the relationship of H-3 and Walter in the development of MH-1 (1). The tests of fruit from the F_1 progeny from Walter × MH-1 indicated that the F_1 was almost as tolerant as the tolerant MH-1 parent (Table 4). There were 50% fewer MH-1 and F_1 fruit with lesions at 5 days after inoculation than were found in Walter. The total decay in fruit from Walter at 15 days after inoculation was significantly greater than that in those of the F_1 or MH-1. The index of survival method of evaluating tolerance produced similar results.

Tests to assess the number of genes involved in inheritance were undertaken during two seasons. In the first season, fruit from individual plants of the parents, the F_1 and the F_2 of a MH-1 × H-3 cross were tested for tolerance to *E. carotovora*. The average percentage of fruit with soft rot within each test grouping indicated that a significant difference in the index of survival existed between the tolerant parent, H-3, and the less tolerant parent, MH-1, while the F_1 was more tolerant than the F_2 (Table 5). Frequencies of the different index of survival ratings of the fruit from individual F_2 plants sampled were compared to the expected frequencies for a normal curve with similar variance and mean (Fig. 1-b). The chi-square value (goodness-of-fit test) equalled 17.39 which fell between $P_{0.10} = 17.3$ and $P_{0.05} = 19.7$.

The next-season fruit from an F_2 population of Walter × H-3, H-3 × Walter, MH-1 × H-3, H-3 × MH-1, and MH-1 × Walter were tested for the frequencies of different levels of the index of survival as described above. A statistical test for homogeneity of the data (6) for the reciprocal cross H-3 × Walter and Walter × H-3 indicated that no difference existed. The data for each of the reciprocal crosses were combined and tested for goodness of fit with a normal curve. The chi-square value for the combined Walter × H-3 reciprocal cross was 11.28, which was bracketed by $P_{0.75} = 10.2$ and $P_{0.50} = 13.3$ (Fig. 1-a). The value for the Florida MH-1 × Walter cross was 6.09, which fell between $P_{0.90} = 7.04$ and $P_{0.95} = 5.98$ (Fig. 1-d). The chi-square value for the reciprocal H-3 × MH-1 cross was 10.12, which was between $P_{0.50} = 7.31$ and $P_{0.25} = 10.20$ (Fig. 1-c).

TABLE 1. Percentage[y] of tomato fruit that developed visible lesions 7 days after being wound-inoculated with four straight pins dipped into inoculum suspension containing 10^6 or 10^7 cells of *Erwinia carotovora* per milliliter and kept at 21 C and approximately 95% relative humidity

Cultivar or line	Season 1		Season 2
	10^6/ml	10^7/ml	10^6/ml
Walter	53 b[z]	42 ab	27 a
Florida MH-1	30 a	28 a	9 b
Heinz-3	8 c	3 c	6 b
Homestead-24	6 c	8 bc	3 b

[y]Average of ten each 10-fruit replicates.
[z]Values within each column not followed by the same letter were different at $P = 0.01$.

TABLE 2. Total percentage[x] and adjusted percentage[y] of tomato fruit that developed lesions 15 days after being wound-inoculated with four straight pins dipped into inoculum suspension containing 10^6 or 10^7 cells of *Erwinia carotovora* per milliliter and kept at 21 C and approximately 95% relative humidity

Cultivar or line	Total	Adjusted decay
Walter	89 a[z]	67 a
Florida MH-1	68 c	30 c
Heinz-3	72 bc	16 d
Homestead-24	84 ab	53 b

[x]Average of ten each 10-fruit replicates.
[y]All fruit which were ripe at least 3 days before lesions appeared were not considered as having decayed.
[z]Values within each column not followed by the same letter were different at $P = 0.05$.

TABLE 3. Average number of pectolytic bacteria present in wounds[y] in tomato fruit at different intervals after inoculation. The fruit were wound-inoculated with four straight pins dipped into inoculum suspension containing 10^7 cells of *Erwinia carotovora* per milliliter and were kept at 21 C and approximately 95% relative humidity

Line	0 hrs	18 hrs	25 hrs	48 hrs	76 hrs
	(×10^2)	(×10^4)	(×10^5)	(×10^6)	(×10^6)
Florida MH-1	9.6 a[z]	11.9 b	2.7 a	1.1 a	12.1 a
Walter	19.0 a	30.0 a	11.7 a	5.1 a	13.0 a
Heinz-3	16.9 a	8.9 b	2.6 a	0.7 a	10.1 a

[y]Average of one wound (four pin holes approximately 2 mm deep) from each of five fruit.
[z]Values within each column not followed by the same letter were different, $P = 0.01$.

TABLE 4. Average percentage[x] of tomato fruit with macroscopic lesions at given intervals after inoculation with four straight pins dipped into inoculum suspension containing 10^7 cells of *Erwinia carotovora* per milliliter

	Total decay		Adjusted decay[y]	Index of survival
	(5 Days)	(15 Days)	(15 Days)	
Walter	26 a[z]	95 a	53 a	9.06 a
Florida MH-1	13 b	84 b	27 b	11.97 b
F_1 (Walter × Florida MH-1)	13 b	76 b	32 b	11.96 b

[x]Average of ten each 10-fruit replicates.
[y]All fruit which were ripe at least 3 days before visible lesions appeared were not considered to have decayed.
[z]Values within each column not followed by the same letter were different at $P = 0.01$.

TABLE 5. Index of survival and percentage of tomato fruit with lesions five days after being wound-inoculated with four straight pins dipped into inoculum suspension containing 10^7 cells of *Erwinia carotovora* and held at 21 C and approximately 95% relative humidity

Line or culture	5 Days	Index of survival
Heinz-3[v]	5 a[z]	11.65 a
Florida MH-1[w]	16 a	8.74 bc
F_1 (Fla. MH-1 × Heinz-3)[x]	14 a	9.66 ab
F_2 [(Fla. MH-1 × Heinz-3) × (Fla. MH-1 × Heinz-3)][y]	19 a	7.98 c

[v]Average of four plants.
[w]Average of 10 plants.
[x]Average of seven plants.
[y]Average of 58 plants.
[z]Values within each column not followed by the same letter were different at $P = 0.05$.

DISCUSSION.—Low populations of *E. carotovora* (approximately 400 bacteria per pin-prick wound) did not immediately initiate soft rot in tomato fruit tissue, nor was the time predictable when a macroscopic lesion would appear. Wound-inoculated fruit of Homestead-24 and MH-1 developed visible lesions at a slower rate than did fruit of other cultivars (1, 2). Once visible lesions had formed, however, further development was so rapid that fruit with lesions had to be separated from healthy fruit within 48 hours to prevent secondary spread of the causal agent.

Studies of the population dynamics of *E. carotovora* in pin-prick wounds in tomato fruit during the 72 hours before visible lesions appeared revealed that the population of bacteria was significantly higher at 18 hours after inoculation in Walter fruit than in H-3 or MH-1. By 76 hours after inoculation, however, there were no apparent differences. Since lesions normally began appearing 72 hours after inoculation, tolerance to soft rot in fruit of different tomato cultivars apparently involved the length of the latent state sometimes exhibited by populations of *E. carotovora*. This situation seemed analogous to the population dynamics of *E. carotovora* in potato lenticels, where viable populations were often present, but only began causing soft-rot when the tubers were placed under anaerobic conditions (7). Inoculated tomato fruit have been stored at 21 C for up to 3 weeks, and no visible lesions appeared. Analysis of the wounds in those fruit for the presence of *E. carotovora* revealed high populations of pectolytic bacteria.

Once a macroscopic soft-rot lesion had formed in a fruit of a tolerant line, however, further development was as fast as that in a susceptible line. The population of *E. carotovora* associated with the beginning of soft rot in tomato fruit was found to be 10^9 to 10^{10} cells/ml by Stall and Hall (8). Assuming that the diameter of the area involved with each 2 mm^2 wound in this study was approximately 0.5 mm, the population present at 76 hours would have been approximately 10^{10} cells/ml, which was about the same level that was present in tissue which was still firm enough to be sectioned in the Stall and Hall test. The population of *E. carotovora* in a developing lesion would probably be higher. Failure of tolerance (as expressed by the rate of development of visible lesions) to correlate with a reduced rate of rotting indicated that tolerance to soft rot in tomato fruit was in reality a tolerance to populations of *E. carotovora* below those necessary to cause soft rot in tissue.

The rate at which visible lesions form in inoculated fruit can be affected by factors other than cultivar tolerance. Studies by the authors (Bartz et al., *unpublished*) showed that such factors include the environment in the storage room, the rate and ratio of nutrients available for fruit development, and the stage of maturity of the fruit at the time of inoculation. In the studies reported here the environment within the storage chamber and in the field were maintained as uniform as possible for the different lines used, but slight variations in maturity of green fruit could not be avoided. Lesions developed faster in ripe or pink fruit than in green fruit.

The variations in the maturity of green fruit were taken into account by two new rating indices. The first system, the adjusted-decay method considered any fruit healthy that would probably have been consumed if it had been in a normal commercial handling and marketing situation. The second, the index-of-survival method, gave a single value to each fruit depending on the maturity of that fruit when soft rot appeared. The latter system was biased against fruit which developed lesions while still green or pink and allowed objective evaluation of the levels of tolerance present after almost all fruit had decayed.

The three methods for evaluation of tolerance of tomato fruits to *E. carotovora* employed indicated that the fruit of an F_1 from Walter × MH-1 were as tolerant as those of MH-1, and that both were significantly more tolerant than Walter. This suggested that tolerance to *E. carotovora* was dominant and not maternally inherited. The F_1 plants used in this test were from several separate Walter × MH-1 crosses, so the chance that the tolerance of the F_1 was due to higher-than-average tolerance for either parent plant seems remote.

The dominance of tolerance to *E. carotovora* was again suggested by the index-of-survival ratings of H-3, MH-1, and the F_1 and F_2 of a MH-1 × H-3 cross. The average of values from the F_1 plants was higher than that from the less-tolerant parent, and was significantly higher than that of the F_2. In addition, the distribution of the index of survival values for the individuals of the F_2 population were skewed toward greater tolerance. The general shape of the distribution curve suggested that tolerance to *E. carotovora* was polygenically controlled.

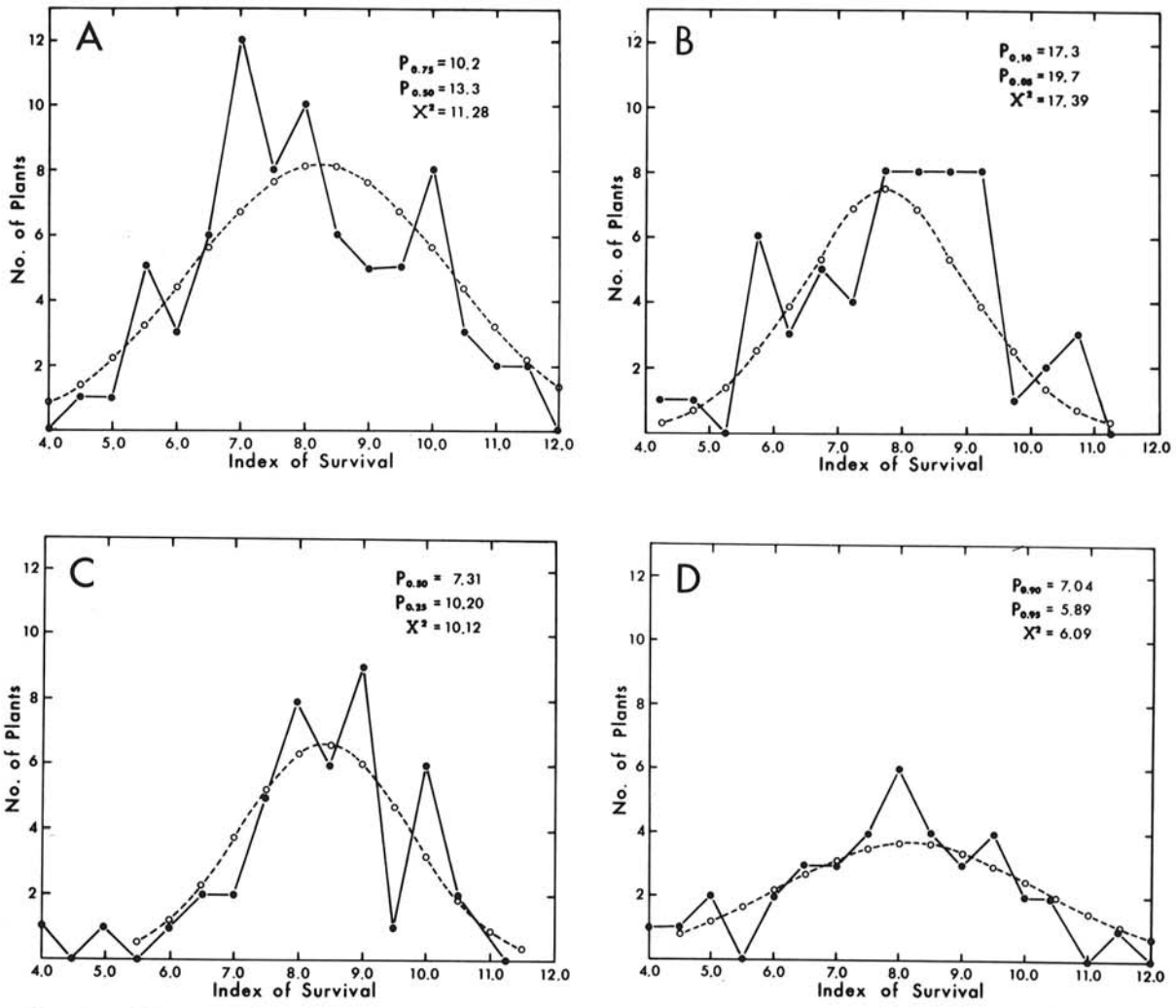

Fig. 1-(A to D). Comparison of tolerance to *Erwinia carotovora* (index of survival) of fruit of F_2 plants from various tomato crosses with a normal curve with similar mean and variance: **A)** Heinz-3 × Walter and Walter × Heinz-3 combined; **B)** Heinz-3 × Florida MH-1; **C)** Heinz-3 × Florida MH-1 and Florida MH-1 × Heinz-3 combined from a different season; **D)** Florida MH-1 × Walter.

The following year the F_2 progeny from H-3 × Walter, Walter × H-3, H-3 × MH-1, MH-1 × H-3, and MH-1 × Walter crosses were tested for tolerance to *E. carotovora*. The distribution of tolerance within each F_2 generation generally supported earlier conclusions. Neither maternal nor paternal inheritance was involved. Inheritance seemed to be polygenically controlled. The distribution of tolerance in the F_2 population from the crosses of Walter and H-3 or MH-1 and Walter fit a normal distribution with high probability (P = .75 to .90) while the distribution of the F_2 from the H-3 and MH-1 crosses fit a normal curve with $P = 0.25$.

There were, however, two interrelated problems associated with the interpretation of these data. The first was that the distribution of index-of-survival values for homozygous plants grown in a uniform environment was unknown and would be impossible to obtain. Thus, a totally objective analysis of the capability of the index-of-survival rating system to detect small but real differences in tolerance to *E. carotovora* was unavailable. However, the variance of the index of survival for a group of MH-1 fruit did prove to be highly significantly greater (P = 0.005) than that of a group of similarly treated Walter fruit. MH-1 should be much more heterozygous with respect to tolerance than Walter, since the latter did not appear to be very tolerant and was developed with one more single-plant selection than was MH-1 (3, 9). The greater variance in the levels of tolerance expressed by individual fruit of a population of MH-1 was expected. From that test we have concluded that the index of survival was a fairly sensitive system for measuring tolerance to *E. carotovora* in tomato, and that the distribution patterns in the F_2 population of the crosses mentioned in this report were not due to variations in the test system.

The second problem was the heterozygosity of MH-1 with respect to tolerance to soft rot, and the extent of the heterozygosity of H-3 with respect to tolerance. The

progeny from crosses made with MH-1 or H-3 could be more or less tolerant, depending on the particular plants used to make the crosses. This made analysis of the dominance of tolerance difficult. H-3 tomato fruit had equal or greater variance with respect to index of survival than those of MH-1. This suggested that selections should be made within H-3 for tolerance to *E. carotovora,* since even higher levels of tolerance than those reported here might be present.

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