Relation Between Resistance of Tomato Fruit to Infiltration by *Erwinia carotovora* subsp. *carotovora* and Bacterial Soft Rot

J. A. BARTZ, Plant Pathology Department, University of Florida, Gainesville 32611

ABSTRACT

Bartz, J. A. 1991. Relation between resistance of tomato fruit to infiltration by *Erwinia carotovora* subsp. *carotovora* and bacterial soft rot. Plant Dis. 75:152-155.

Fruit of some tomato cultivars, including Sunny and Horizon, did not absorb water or cell suspensions of *Erwinia carotovora* subsp. *carotovora* as readily as did those of Florida MH-1, Walter, or Flora-Dade. These differences were consistent among fruit from successive harvests of the same crop or harvests of different crops in the same or different years. Large and mature green fruit absorbed more water or inoculum than did small, pink ones. The incidence of bacterial soft rot among infiltrated fruit increased with quantity of inoculum absorbed and was unrelated to disease development in wound-inoculated fruit. The resistance of some cultivars to bacterial soft rot as determined through wound-inoculation tests was overcome by the volume of inoculum absorbed when fruit were inoculated by infiltration. Some significant differences among cultivars for disease incidence persisted, however, even though incidence was adjusted to account for differences in inoculum absorbed. Therefore, some characteristic of tomato fruit besides porosity of the stem scar to water affected disease development. Fruit that resist infiltration by water are less likely to become inoculated with rot-inducing fungi or bacteria when handled in the water dumps and flumes at modern packinghouses.

Resistance in mature green fruit of tomato (*Lycopersicon esculentum* Mill.) to bacterial soft rot was associated with a slower multiplication of *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey et al in wounded tissues, which prolonged the latent period of the disease (2,5,6). High populations of the pathogen were isolated from inoculated wounds but most lesion development was delayed until after fruit became fully red (2,6). Such fruit could be marketed and consumed without evidence of the disease.

In commercial practice, postharvest losses to bacterial soft rot are not always associated with wounded fruit (1). When fruit are submerged in water during handling and harvesting operations, stem scar tissues or open blossom pores can become infiltrated with aqueous suspensions of *E. c.* subsp. *carotovora* or other postharvest pathogens (1,3,4,7). Infiltrated fruit usually decay rapidly. Chlorination of water used to infiltrate fruit contaminated with *E. c.* subsp. *carotovora* led to less disease but also to greater water intake than with the control treatment (4). Unacceptable disease incidences developed among fruit infiltrated with chlorinated water.

Fruit inoculated by infiltration are more likely to become diseased than are those subjected to wound inoculation. Stem scar tissues can absorb milliliter volumes of inoculum (3,7) that may permeate much of the white connective tissue in the center of a fruit (Bartz, *unpublished*). Punctures on fruit surfaces that would escape detection and culling at packinghouses, such as those made by grains of sand, were estimated at <2 mm in diameter and depth (3). The volume of these wounds, about 2 μl, is nearly 500-fold less than volumes absorbed by infiltrated fruit (3,4,7).

The amount of infiltration has varied among different fruit lots and individual fruit of the same lot (3,7). Some fruit did not absorb a detectable volume (0.1 ml), whereas others absorbed nearly 1.0 ml (3). Fruit whose stem scars resist water intrusion would not be readily inoculated when handled through the water dumps and flumes of modern packinghouses. Such fruit would be desirable in commercial handling. Impervious stem scar tissues or closed blossom pores would effectively exclude all postharvest pathogens that become suspended in water at packinghouses.

In the tests described herein, fruit were infiltrated with water or cell suspensions of *E. c.* subsp. *carotovora* to find if consistent differences existed among cultigens (breeding lines and cultivars) for water absorption and if disease incidence among infiltrated fruit depended on the quantity of inoculum absorbed.

Florida Agricultural Experiment Station Journal Series No. R00637.

Accepted for publication 16 July 1990 (submitted for electronic processing).

MATERIALS AND METHODS

Fruit samples. Mature green or light pink fruit were hand-harvested from observational or replicated yield trials in experiment station plots or commercial fields and then selected for freedom from obvious defects. All cultigens were grown with standard commercial cultural practices. The fruit were transported to the laboratory and treated within 48 hr after harvest.

Infiltration treatments. In the initial tests, fruit were infiltrated by submersion for 10 min in an aqueous cell suspension that was 17° cooler than fruit pulp temperature (7). In subsequent tests, fruit were submerged in water or a cell suspension in a pressure cooker and exposed to an air pressure that forced 0.5–1.0 ml of water into stem scars of one of the cultivars, typically those of Florida MH-1 (3). The pressures, expressed as immersion depth, ranged from 91 to 122 cm for 1–2 min. All fruit within a test were exposed to the same treatment and were weighed, either individually or in groups of five, before and after treatment. Fruit surfaces were dried with a cotton towel before the final weighing. Infiltration was based on the increase in fruit weight due to treatment.

Inoculation. Aqueous cell suspensions of *E. c.* subsp. *carotovora* were prepared from 24-hr nutrient broth shake cultures (2,5). The cells were harvested by centrifugation; pellets were suspended in deionized water and diluted to about 1×10^6 cfu/ml. For inoculation by infiltration, the fruit were submerged in the aqueous cell suspension and treated as described above. For wound-inoculation, fruit were wounded four times with four straight pins that had been dipped into the cell suspension (2,5). Each fruit had 16 2 × 2 mm punctures.

Disease development after inoculation by infiltration or wound contamination. Sixty fruit were harvested from each of five cultivars grown at the Southwest Florida Research and Education Center. Thirty fruit were separated into 10-fruit replicates, warmed to 37 C, and infiltrated with an aqueous cell suspension of *E. c.* subsp. *carotovora* at 20 C. The second set of 30 fruit was separated into replicates and wound-inoculated. The fruit were stored at 20 C. In a second test, 50 fruit were harvested from the same cultivars grown in a nearby commercial field. Two 10-fruit replicates were infiltrated and the remainder were wound-inoculated. These fruit were stored at 26 C to increase the rate of disease development. Disease incidence was recorded after 3 days for all treatments in both tests. The data for both tests were combined and analyzed as a

split plot; cultivar was the main plot and test (storage temperature and field) was the subplot factor.

Infiltration of fruit from different cultivars by submersion as affected by successive harvests of the same plants. Fruit were harvested three times as per commercial practice from the plants grown in observational trials at the Gulf Coast Research and Education Center in the springs of 1983 and 1984. Ten fruit were harvested from each of 10 cultigens on 26 May and 3 and 10 June 1983. The fruit were pressure-infiltrated with tap water as described above. There were 10 single-fruit replicates for each cultivar at each harvest, and harvest was treated as a subplot factor in a split-plot-in-time design. In the second year, 15 fruit were harvested from each of three field-plot replicates arranged in a randomized complete block. Harvest dates were 23 May and 1 and 14 June. The fruit were treated in groups of five. Weight increases were calculated for each five-fruit unit. The mean of the three units from each plot was used in statistical analysis of the split-plot-in-time design.

Additional tests were performed over the winter of 1983–84 on fruit obtained from variety trials in Homestead. In each test, the fruit were from a single harvest. The fruits in one test had to be sorted by color (green or pink).

Infiltration of fruit from different cultivars as affected by fruit ripeness. Fifty fruit were harvested from each of 10 cultigens grown in a observational trial in a commercial field. Forty fruit were selected, separated by color (green or pink), and, in five-fruit units, submerged in an aqueous cell suspension of *E. c.* subsp. *carotovora*. After the weight increase was recorded, the four five-fruit units were combined into two 10-fruit replicates, stored at 20 C, and evaluated daily for disease incidence over a 5-day period.

Statistical analyses. The data were evaluated by Statistical Analysis System (SAS) system software either at the Northeast Regional Data Center at the University of Florida or with SAS for the PC. Programs used included the ANOVA procedure for balanced designs; the GLM procedure for unbalanced designs, including those with unequal replication or missing data; and the GLM procedure for analysis of covariance and least square means (8). The covariance test, a combination of regression analysis and analysis of variance, was used to determine if fruit size affected the amount of infiltration. The least square means test was used to adjust cultigen means to account for differences in fruit size. The incidence of disease expressed as percentages was transformed by the arcsine square root method; the weight increase of fruit due to infiltration, expressed as the (weight increase/initial weight) × 100, was transformed by the square root of (0.5 + percent weight increase). Bartlett's test for the homogeneity of variances was performed as described by Steel and Torrie (10). The levels of significance reported herein are * = 0.05, ** = 0.01, *** = 0.001, and **** = 0.0001; probabilities greater than 0.05 were considered not significant.

RESULTS

Comparison of cultivars for level of infiltration and disease development after infiltration or wound-inoculation. In two separate tests, fruit of Florida MH-1 were infiltrated with largest quantity of inoculum and had the highest incidence of disease (Table 1). In contrast, fruit of Burgis developed the most disease after wound-inoculation. The cultivars differed for weight increase because of infiltration (F = 39.7****), disease after infiltration (F = 22.8***), and disease after wound-inoculation (F = 5.3*). Despite differences in fruit size (152 vs. 197 g per fruit) and absorption of inoculum (0.12 vs. 0.48 g per fruit) between the tests, the cultivars ranked similarly in each test for weight increase. The use of higher incubation temperatures in the second than in the first test led to increased disease after infiltration (10 vs. 46%, F = 668.8****) or wound inoculation (4 vs. 39%, F = 48.1****), but the ranking of the cultivars for these parameters was not affected (*data not shown*). The interaction cultivar × test was not significant for disease after wound inoculation, whereas a small but significant interaction between test and cultivar for disease after infiltration (*data not shown*) did not affect the cultivar ranking.

The absorption of water by fruit increased with sample weight, which was a significant covariable (F = 38.2****) in the effect of cultivar on absorption. The expression of weight increase as a percentage of sample weight reduced but did not eliminate the significant covariance of sample weight. The interaction of sample weight with cultivar, the slope of the regression of weight increase over sample weight, was not significant. Thus, sample weight affected absorption similarly for each cultivar. Cultivar adjusted for sample weight also affected infiltration significantly (F = 14.3****). When the percent weight increase for each cultivar was adjusted to account for the effect of sample weight (least square means test), means for the smaller samples were higher than the measured value, whereas those for the larger samples were lower.

Disease among infiltrated fruit increased with quantity of inoculum absorbed. For disease incidence at 3 or 5 days after infiltration, the percent weight increase was a significant covariable in the effect of cultivar on disease incidence, F = 7.73** or 13.29**, respectively. The slope term in the analysis of covariance, cultivar × sample weight, produced a small F value ($P > F > 0.2$). Thus, the correlation between inoculum absorption and disease development was similar for every cultivar. All variation in disease incidence at the 3-day observation was accounted for by the amount of infiltration (F for cultivar = 1.01^{ns}), whereas at 5 days, cultivar accounted for a portion of the variation (F = 3.15*). Thus, the percent weight increase was the most important factor for disease incidence after inoculation by infiltration, whereas the apparent resistance of the tissues to disease had little influence.

Effect of successive harvests of the same plants on ranking of cultivars for absorption of water. In tests of fruit from three harvests of the same plants conducted in two separate years, fruit of Florida MH-1 absorbed more water than did those of any other cultivar (Table 2). In the first year, the average size of the fruit decreased over the three harvests, from 164 to 140 to 138 g per fruit, whereas water uptake increased from 0.25 to 0.34% of the original sample weight. The cultigens (eight cultivars and two advanced breeding lines) differed for percent weight increase (F = 16.76****); there was a small but significant (F = 2.18**) interaction between cultigen and harvest, however. This interaction occurred because of shifts in the ranking of three cultivars, e.g., Walter ranked first, fifth, and second in the three tests, respectively, whereas Duke ranked seventh, third, and third, respectively, and FTE12 was fourth, ninth, and tenth, respectively. Use of individual fruit as replicates

Table 1. Incidence of bacterial soft rot in tomato fruit of five cultivars after inoculation by infiltration or contamination of wounds[w]

Cultivars	Infiltration[x] Percent weight increase	Percent disease	Percent disease in wounds[y]
Florida MH-1	0.34 a[z]	60 a	12 b
Flora-Dade	0.10 b	18 bc	13 b
Hayslip	0.10 b	16 bc	12 b
FTE12	0.10 b	20 b	18 b
Burgis	0.09 b	10 c	47 a

[w] Fruit at 37 C were submerged in 1 × 10^6 cfu/ml of *Erwinia carotovora* subsp. *carotovora* at 20 C for 10 min. Four straight pins protruding 2 mm through a cork were dipped into a suspension of *E. c.* subsp. *carotovora*, then pressed against the side of a fruit. Each fruit was wounded four times for a total of 16 punctures. Inoculated fruit were incubated for 3 days at 20 and 26 C in the first and second of two separate tests, respectively.

[x] Each value is the average of five 10-fruit replicates, three in the first and two in the second test.

[y] Each value is the average of three 10-fruit replicates in each of two separate tests.

[z] Values within each column not followed by the same letter were different at P = <0.05 in the Waller-Duncan multiple range test.

was not entirely satisfactory because field effects were not included in the model and the R^2 for the model was only 0.59. However, the sample variances were homogeneous based on Bartlett's test (10).

In the second year, fruit sizes were larger in the second than in the first harvest—163, 181, and 157 g per fruit, respectively (F = 19.10****)—but the percent weight increases were similar over harvest, as the latter did not affect infiltration. The cultigens differed for percent weight increase (F = 13.4****) but ranked similarly for water absorption within each harvest (the cultigen × harvest interaction was not significant).

The analysis of covariance for the effect of cultivar on the percent weight increase produced results similar to those in previous tests, e.g., sample weight and cultigen terms were significant, whereas the slope term (sample weight × cultigen) was not. Thus, sample size and cultigen affected infiltration, whereas the effect of sample size was similar over cultigen. When cultivar means were adjusted for differences in sample weight, again the percent weight increases for cultivars producing lighter samples were adjusted upward and those for the heavier ones downward.

Effect of fruit ripeness on cultigen ranking for absorption of inoculum and development of disease. The cultigens differed in their absorption of inoculum (F = 12.55****), fruit size (182–225 g per fruit, F = 6.09****), and development of disease (at 3 and 5 days after inoculation, F = 4.18* and 4.86*, respectively) (Table 3). The ranking of the cultigens in order of inoculum absorption or disease was similar at each stage of ripeness (F for interaction of either cultivar or disease with ripeness was not significant). The pink fruit were larger than the green ones (204 vs. 191 g per fruit, F = 23.9****) but absorbed less inoculum (0.18% vs. 0.23%, F = 9.94**).

Sample weight was a significant covariable in the effect of ripeness on percent weight increase (F = 21.53****), whereas the interaction of sample weight with ripeness was not significant. Thus, the percent weight increase in each ripeness category was affected similarly by sample weight. In contrast, the transformation of weight increase to a percentage of sample weight eliminated the contribution of sample weight to the variation in infiltration among the cultigens.

The ranking of the cultivars for disease incidence was generally correlated with that for absorption, but with exceptions (Table 3). Fruit of BL4049 were the largest, absorbed the most inoculum, and ranked second for disease incidence. Fruit of BL7129, however, had a similar amount of disease but absorbed significantly less inoculum. Thus, some factor besides quantity of inoculum absorbed was involved with development of disease. This conclusion was supported by results of a test of the covariance of percent weight increase with cultigen on disease at 2–5 days after inoculation. At 2 days after inoculation, neither cultigen nor weight increase affected disease (F = 1.08^{ns} and 1.11^{ns}, respectively), but at 4 and 5 days, both did (at 4 days F = 5.58* and 3.22**, respectively, and at 5 days F = $3.28^{0.081}$ and 2.87**, respectively).

The cultivars in the test—Duke, FTE12, Sunny, and Horizon—were ranked similarly for absorption as in previous tests, e.g., Duke absorbed the most, followed by FTE12, Sunny, and Horizon. The latter two were not significantly different.

The effect of ripeness in the above test was similar to that observed in a Homestead test (*data not shown*), e.g., the riper fruit absorbed less water but ripeness did not interact with cultigen in the evaluation of cultigens for water absorption. In the latter tests, the differences among cultigens for water absorption were consistent with those reported above, e.g., Duke, FTE12, and Flora-Dade were intermediate for water absorption, and Sunny or Horizon absorbed significantly less water.

DISCUSSION

The absorption of water, even if chlorinated (4), from dump tanks and flumes at modern packinghouses can be the primary factor leading to postharvest diseases in tomato fruit (1). In the tests reported here, consistent differences occurred among cultigens in absorption of water by submerged fruit despite variation in uptake associated with sample weight, successive harvests, field plot, and stage of ripeness.

Although the absorption of the initial increment of inoculum is the key factor in the development of disease, the relative amount of water absorbed (= percent weight increase) was the pertinent dependent variable for comparison of cultigens for resistance to infiltration. Theoretically, larger fruit could absorb more water than smaller ones because of larger stem scars and more connective tissue beneath stem scars. Indeed, in each of the tests reported here, sample weight was a significant covariable in the effect of cultigen on weight increase. The conversion of the latter to a percentage of sample weight eliminated the significant covariance of the sample in two of the tests. Because each sample contained the same number of fruit, signif-

Table 2. Percentage of weight increase due to water absorption by submerged fruit harvested from different tomato cultigens in two seasons[w]

Cultigen	Percent weight increase	
	1983[x]	1984[y]
Florida MH-1	0.69 a	0.56 a
Walter	0.48 ab	0.37 b
Flora-Dade	0.33 bc	0.25 c
Duke	0.33 bc	...[z]
Suncoast	0.32 bc	...
Hayslip	0.23 cd	0.23 c
BL7025	0.22 cd	0.37 b
FTE12	0.21 cd	...
Sunny	0.16 cd	...
BL7057	0.09 d	0.21 c
Horizon	...	0.21 c

[w] Fruit were submerged in water in a pressure cooker and exposed to an air pressure equivalent to immersion to a depth of 122 cm for 2 min in 1983 and for 1 min in 1984. Before statistical analyses, percentage weight increase values were transformed by the square root of 0.5 + X, where X = weight increase/original weight.

[x] Each value is the percent weight increase for three 10-fruit samples (10 fruit harvested from the same plants on three different dates). Values within each column not followed by the same letter were different at P = 0.05 in the Waller-Duncan multiple range test.

[y] Each value is the percent weight increase for nine 15-fruit samples harvested from three field replications on three different dates.

[z] Not available or not tested.

Table 3. Disease incidence and water uptake characteristic of fruit harvested from 10 cultigens and inoculated by submersion in an aqueous cell suspension of *Erwinia carotovora* subsp. *carotovora*[y]

Cultigen	Sample weight (g/fruit)	Weight increase		Percent disease
		g/fruit	%	
BL4049	222 a[z]	0.86 a	0.38 a	96 ab
Duke	193 cdef	0.56 ab	0.29 ab	81 bc
BL7175	202 bcd	0.54 bc	0.27 bc	81 bc
BL7129	206 b	0.50 bc	0.24 bcd	99 a
BL7177	204 bc	0.42 bcd	0.21 bcde	79 c
BL7131	195 bcde	0.36 cd	0.19 cde	78 c
FTE12	188 ef	0.34 cd	0.18 de	70 cd
BL7155	192 def	0.26 de	0.13 ef	53 d
Horizon	194 cdef	0.18 e	0.09 f	58 cd
Sunny	182 f	0.14 e	0.08 f	58 cd

[y] Fruit were separated by color (green or pink), submerged in an aqueous cell suspension of *E. c.* subsp. *carotovora* at 10^6 cfu/ml, and immersed to a depth of 91 cm for 2 min. Four five-fruit replicates were used for absorption; for percent disease, the samples were combined into two 10-fruit replicates. Inoculated fruit were stored at 20 C for 4 days.

[z] Values within each column not followed by the same letter were different at P = <0.05 in the Waller-Duncan multiple range test.

icant differences for sample weight meant differences in fruit weight. Unless cultigens in a comparison yielded similar-sized fruit or unless fruit harvested from those cultigens were of similar size, differences in average fruit size could bias the comparison. Use of the least square means test adjusted means for infiltration to account for differences in fruit size. When water uptake was adjusted for fruit size, average weight increases (*data not shown*) in each of the four tests were higher than the measured amount for the cultigens represented by smaller fruit and lower than that measured for those represented by larger fruit. The same relationship was found when water uptake was expressed as a percentage of sample weight. Thus, at the level of infiltration observed here, larger fruit absorbed proportionately more water than did smaller ones. The potential bias associated with sample weight must be considered in all comparisons of cultigens for resistance to infiltration.

Disease incidence among the infiltrated fruit usually varied with the amount of inoculum absorbed. Fruit that absorbed the largest quantity were expected to develop the most disease. Indeed, the weight increase due to infiltration was a significant covariable for the effect of cultivar on disease in most covariance tests. Fruit that absorb measurable inocula usually decay (3,7). When disease incidence was adjusted for inoculum absorbed, however, a few statistically significant differences for disease among cultigens were observed. Thus, some fruit characteristic other than stem scar porosity affected disease development. This characteristic did not appear related to the susceptibility of the tissue to bacterial soft rot because disease after wound-inoculation was not related to that after infiltration. If cultivar resistance to bacterial soft rot expressed in wound-inoculated fruit is a resistance in the fruit tissues to bacterial soft rot (2,6), then that resistance in the tests reported here was overridden by the volume of inoculum absorbed by infiltrated fruit. For example, in several different wound-inoculation tests, fruit of Florida MH-1 appeared to possess resistance to the disease (5,6). In the tests reported here, however, fruit of Florida MH-1 consistently absorbed the most water and developed the most disease. Moreover, adjustment of the average disease incidence among fruit of Florida MH-1 to account for differences in inoculum absorption did not alter its ranking as the most susceptible cultivar.

Resistance in tomato fruit to infiltration is potentially a valuable cultivar characteristic that should be described in release bulletins (9). Fruit with porous stem scars such as those of Florida MH-1 may require special handling at packinghouses. In contrast, as part of a strategy for controlling postharvest diseases, growers should be able to select cultivars that produce fruit with stem scars that resist water intrusion.

LITERATURE CITED

1. Bartz, J. A. 1980. Causes of postharvest losses in a Florida tomato shipment. Plant Dis. 64:934-937.
2. Bartz, J. A. 1981. Variation in the latent period of bacterial soft rot in tomato fruit. Phytopathology 71:1057-1062.
3. Bartz, J. A. 1982. Infiltration of tomatoes immersed at different temperatures to different depths in suspensions of *Erwinia carotovora* subsp. *carotovora*. Plant Dis. 66:302-306.
4. Bartz, J. A. 1988. Potential for postharvest disease in tomato fruit infiltrated with chlorinated water. Plant Dis. 72:9-13.
5. Bartz, J. A., and Crill, J. P. 1972. Tolerance of fruit of different tomato cultivars to soft rot. Phytopathology 62:1085-1088.
6. Bartz, J. A., Crill, J. P., and John, C. A. 1975. Inheritance of tolerance to *Erwinia carotovora* in Florida MH-1 tomato. Phytopathology 65:1146-1150.
7. Bartz, J. A., and Showalter, R. K. Infiltration of tomatoes by bacteria in aqueous suspension. Phytopathology 71:515-518.
8. Freund, R. J., and Littel, R. C. 1981. SAS for Linear Models, a Guide to the ANOVA and GLM Procedures. SAS Institute Inc., Cary, NC. 231 pp.
9. Scott, J. W., Olson, S. M., Bryan, H. H., Howe, T. K., Stoffella, P. J., and Bartz, J. A. 1989. Solar Set, a heat tolerant, fresh market tomato hybrid. Fla. Agric. Exp. Stn. Circ. S-359. 10 pp.
10. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York. 481 pp.