Tolerance of Fruit from Different Pepper Lines to Erwinia carotovora

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ABSTRACT

Fruit from different pepper lines were not equally susceptible to *Erwinia carotovora*. Only 3% of the fruit of the most tolerant cultivar, Jalapeno, had lesions 2 days after inoculation, whereas 75% of the fruit of the most susceptible cultivar tested had lesions. All inoculations were made by wounding the fruit with straight pins that had been dipped in a suspension of *E. carotovora*. Tolerance was inherited, as indicated by assays of progenies from two test crosses, and was controlled by a relatively few genes, or a few groups of linked genes.

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Bacterial soft rot, caused by *Erwinia carotovora* (L. R. Jones) Holland, is a common postharvest problem of bell peppers, *Capsicum annuum* L., grown in Florida. Fruit decay usually begins at wounds in the walls (carpels) or stems (peduncles).

Prevention of decay in transit has been a major concern. Near-freezing temp cannot be used to prevent decay in transit, because peppers, like tomatoes, are susceptible to chilling injury (4). Bartz and Crill (1, 2) reported a delay in symptom expression in inoculated fruit from certain tomato cultivars. This delay was viewed as an expression of tolerance to *E. carotovora*, and helped insure successful shipment of tomatoes to terminal markets.

A similar delay in symptom expression in inoculated peppers of certain cultivars was recently noted (5). In that study, the fruit were inoculated by atomizing suspensions of *E. carotovora* onto freshly wounded stems or over the wounds caused by stem removal. The following reports on the relative tolerance to *E. carotovora* possessed by fruit from certain pepper lines that were uniformly wound-inoculated.

MATERIALS AND METHODS.—Pepper fruit used in this study were hand-harvested from plants grown with identical cultural practices during the spring seasons of 1972 and 1973. Only three replicates of each entry were available for tests in 1972, whereas 10 were available in 1973. Almost all replicates contained 10 fruit.

The cultivars used in these tests and their sources were the following: Jalapeno, Anaheim Chili, Red Cherry, Cascabella, Floral Gem, Jade, Early Calwonder-#1, and All Big from the Asgrow Seed Co.; Canape, Large Red Cherry, Hungarian Wax, Penn Bell, Hot Portugal, Cubanelle, and Keystone Resistant Giant from the Harris Seed Co.; Roumanian Sweet from the Leathermans Seed Co.; Canada Cheese, Szegedi, and Shepherd from the Stokes Seed Co.; Yolo Wonder and Early Calwonder-#2 from the Northrup King Seed Co. The variety Avelar was from Dr. A. A. Cook, who had obtained it from Dr. H. Nagai. The breeding line 68-14 was a selection from U.S. Department of Agriculture plant introduction (P.I.) 124540. The breeding line 23-1-7Bk was derived, predominantly from, cultivars Yolo Wonder L and P.I. 163192, and has resistance to one race of bacterial spot, dominant-gene resistance to tobacco mosaic virus, and tolerance to tobacco etch virus and potato virus Y (A. A. Cook, *personal communication*).

Inoculum was prepared from 24-h nutrient broth cultures of *E. carotovora* which were continuously agitated at room temp (75-80 F). The strain (SR-12) used was originally isolated from a partially decayed melon. A pure culture of this organism rotted potato slices, fermented glucose in the Hugh-Leifson test, produced nonfluorescent colonies on King's Medium B, and did not produce oxidase. In addition, this strain did not produce acid from α-methyl glucoside but did produce reducing sugars from sucrose and did grow at 36 C. From the results of these tests, the organism was considered to be *E. carotovora* (3).

Bacteria centrifuged from the cultures were resuspended in sterile buffered saline at pH 7.0 (0.8 g NaCl, 0.2 g NaH_2PO_4 and 0.2 g KH_2PO_4 to one liter in distilled water). The resulting suspension was adjusted to 0.3 optical density in a Spectronic 20 spectrophotometer set at 600 nm. This provided a suspension of ca. 10^8 cells/ml. From that stock suspension, final inoculum suspensions containing 1- or 5×10^6 cells/ml were prepared in the buffered saline.

Each pepper fruit was wounded in four locations spaced equally around its circumference by a wounding instrument composed of four straight pins ca. 5 mm apart piercing a No. 0 cork stopper by ca. 2 mm. The instrument was dipped into the inoculum before each wound was made. When the susceptibility of the calyx and stem were compared to that of the walls, approximately equal numbers of wounds were made in each area of the fruit.

All inoculated fruit were stored on 0.38×0.51 m ($15 \times$ 20-inch) plastic trays at 21 C (70 F) and ca. 95% relative

TABLE 1. Percentage of pepper fruit that developed lesions after being wound-inoculated with 1×10^6 cells of *Erwinia carotovora* per ml buffered saline, and kept at 21 C (70 F) and approximately 95% relative humidity

Cultivar	Days after inoculation[b]		
	3	5	7
Jalapeno[a]	10 BCD	13 E	13 D
Anaheim Chili	13 BCD	33 DE	33 CD
Red Cherry	20 BCD	33 DE	40 BCD
Cascabella	27 BCD	40 CDE	43 ABCD
Canape	13 BCD	40 CDE	47 ABCD
Large Red Cherry	3 D	43 BCDE	47 ABC
Roumanian Sweet	13 BCD	47 ABCD	57 ABC
Canada Cheese	17 BCD	67 ABCD	70 ABC
Fresno Chili	30 ABCD	53 ABCD	57 ABC
Hungarian Wax	37 ABCD	67 ABCD	70 ABC
Penn Bell	37 ABCD	53 ABCD	67 ABC
Hot Portugal	43 ABCD	57 ABCD	73 ABC
Szegedi	43 ABCD	67 ABCD	73 ABC
Bell Boy Hybrid	43 ABCD	70 ABCD	80 AB
Long Thin Cayenne	47 ABC	67 ABCD	70 ABC
Floral Gem	60 AB	77 ABC	80 AB
Cubanelle	77 A	80 AB	80 AB
Shepherd	53 ABC	83 A	83 AB

[a]Average of three 10-fruit replicates.
[b]Values not followed by the same letter are statistically different at $P = 0.05$.

humidity (RH). Fruit with lesions were discarded daily beginning 2 days after inoculation. Decay was expressed as the percentage of fruit having soft rot lesions on the observation date indicated. The percentage data were transformed by the *arc sine square root of percentage* method and analyzed statistically by the Duncan's New Multiple Range Test.

RESULTS.—There were large apparent differences present among the means of the susceptibilities of the different lines in the initial screening (Table 1). Only 3% of the fruit of cultivar Large Red Cherry had lesions 3 days after inoculation with *E. carotovora* (1×10^6 cells/ml) as compared to 77% of the fruit of Cubanelle. By the 5th and 7th day, however, Jalapeno fruit had many fewer soft rot lesions than did Large Red Cherry fruit. Although both Jalapeno and Large Red Cherry were pungent types, tolerance could not be correlated with pungency because the pungent cultivars, Long Thin Cayenne and Floral Gem, ranked among the least tolerant varieties.

For the second test of the tolerance of pepper fruit to soft-rotting bacteria, fewer lines were considered, but larger numbers of fruits were used per line. Only four replicates of 23-1-7Bk were available. The inoculum concn was increased 5-fold in comparison with the preceding test, and lesions appeared 2 days after inoculation.

Jalapeno fruit were significantly more tolerant than those from any other pepper line (including Large Red Cherry) at even the first observation date (Table 2). Expansion of the few lesions in Jalapeno fruit was also much slower than in other fruit. A bell pepper type, Jade, was the most susceptible; 75% of its fruit had lesions only 2 days after inoculation, as compared to just 3% of the Jalapeno fruit which had been similarly treated. Those peppers less tolerant than Jalapeno but more tolerant than Jade fell statistically into two groups: 14-24% of the fruit with lesions, vs. 37-45%. The only overlap between these groups was believed to be due to the few replicates of 23-1-7Bk available. The same general groupings were apparent 1 day later.

Both of the preceding tests were based on the tolerance of the flesh of the fruit. During normal commercial handling of peppers, the calyx and stem as well as the walls of the fruit would be exposed to soft rot inocula. The stem, calyx, and side of fruit of nine different pepper cultivars were inoculated with ca. the same number of wounds to determine if different tolerances to soft rotting bacteria would be expressed by different parts of a pepper fruit. There were five 10-fruit replicates of each entry.

The walls of the pepper fruit were, in general, more susceptible to *E. carotovora* than either the stem or the

TABLE 2. Percentage of pepper fruit that developed lesions after being wound-inoculated with 5×10^6 cells of *Erwinia carotovora* per ml buffered saline and kept at 21 C (70 F) and approximately 95% relative humidity

Cultivar or line	Days after inoculation[b]	
	2	3
Jalapeno[a]	2 A	3 A
Large Red Cherry	14 B	14 B
23-1-7Bk	15 BC	18 BC
Avelar	20 B	26 BC
Yolo Wonder	24 B	28 BCD
Keystone Resistant Giant	37 CD	38 CDE
Hot Portugal	43 D	44 DE
All Big	43 D	48 DE
Early Calwonder #1	42 D	49 DE
Early Calwonder #2	45 D	51 E
Jade	75 E	78 F

[a]Values given were the average of ten 10-fruit replicates except for 23-1-7Bk where only four replicates were available.
[b]Values with each column not followed by the same letter were statistically different at $P = 0.05$.

TABLE 3. Percentage of pepper fruit that developed lesions 2 days after the stem, calyx, and side of each fruit had been wound-inoculated with 5×10^6 cells of *Erwinia carotovora* per ml buffered saline, and kept at 21 C (70 F) and approximately 95% relative humidity.

	Location of Lesions[b]		
	Stem	Calyx	Side
Jalapeno[a]	2 A	0 A	4 A
Large Red Cherry	30 B	26 BC	24 B
Hot Portugal	28 B	18 B	68 C
Avelar	62 BC	42 BCD	70 C
Jade	48 BC	46 CD	84 C
Yolo Wonder	58 BC	46 CD	62 C
Early Calwonder	45 BC	45 BCD	83 C
All Big	73 C	78 C	88 C
Keystone Resistant Giant	60 BC	64 DE	74 C
Averages[c]	45 X	40 X	63 Y

[a]Average of five 10-fruit replicates.
[b]Values within each column not followed by the same letter were statistically different at $P = 0.05$.
[c]Averages not followed by the same letter were statistically different at $P = 0.05$.

calyx (Table 3). However, those three areas of fruit from Large Red Cherry, Yolo Wonder, and All Big were equally susceptible. The tolerance to *E. carotovora* shown by at least one line was affected by the location of the wound. If just those lesions originating at wounds on the sides of fruit were considered, the susceptibility of Hot Portugal fruit was not different from that of All Big, the most susceptible line tested. But, if only stem or calyx lesions were considered, Hot Portugal was significantly more tolerant than All Big.

The progeny of crosses between certain pepper cultivars were available for a preliminary study of the possible inheritance of tolerance to soft-rotting bacteria. One cross which was studied was (23-1-7Bk) × (68-14). The F_2 of this cross comprised four different selections from the F_1 and totalled 34 plants. Two backcrosses (B×) between the F_1 and 23-1-7Bk with a total of 20 plants were also sampled. Ten fruit were harvested from each plant and individually labelled with both line and plant number designations, except for 23-1-7Bk which was an advanced bell-type breeding line. Thirty fruit from 23-1-7Bk were bulked and divided into three 10-fruit replicates.

The second study of the inheritance of tolerance to *E. carotovora* concerned a cross between Jalapeno and 68-14. There were 15 plants of Jalapeno, 22 of 68-14, and 23 of the F_2 plants harvested and handled as described above.

TABLE 4. Average percentage of pepper fruit from different lines developing lesions 2 days after being wound-inoculated with 5×10^6 cells of *Erwinia carotovora* per ml buffered saline

Entry	No. of Replicates	Decay[a]
Test cross 1		
P_1 (23-1-7Bk)	3	7 A
P_2 (68-14)	6	43 C
F_2	34	35 C
Backcross		
$F_1 \times$ 23-1-7Bk	20	19 B
Test cross 2		
P_1 (Jalapeno)	15	8 X
P_2 (68-14)	22	50 Z
F_2	23	37 Y

[a]Values within each test not followed by the same letter were statistically different at $P = 0.05$.

Fruit from 68-14 plants were significantly more susceptible to *E. carotovora* than were either 23-1-7Bk or Jalapeno (Table 4). The average tolerance of the fruit from the F_2 plants in the cross between 68-14 and 23-1-7Bk was not significantly different from that of 68-14. The average of the fruit from the B×'s between 23-1-7Bk and the F_1 (23-1-7Bk × 68-14) was significantly lower than that of 68-14. In the second cross between 68-14 and Jalapeno, the fruit from the F_2 plants were significantly less susceptible than those from 68-14.

The frequency of various levels of tolerance of fruit from the individual plants within each test cross is illustrated in Fig. 1. The reactions of all 68-14 fruit were combined and placed in Fig. 1-B; 23-1-7Bk plants were not included in these graphs because the low number of fruit harvested per plant would not allow an accurate determination of the tolerance of fruit from each plant within the test. The graphs illustrate that three levels of tolerance existed in these crosses. The F_2 plants in both crosses seemed to be divided into two groups with respect to tolerance to *E. carotovora*. The Jalapeno parental level of tolerance was apparently not found in the F_2 plants. Backcrossing the F_1 from the first test cross with 23-1-7Bk seemed to eliminate the 68-14 level of tolerance.

DISCUSSION.—Tolerance to soft rot in the fruit of tomato, and now pepper, has been demonstrated here and in an earlier report (1, 2, 5). This tolerance is expressed as a reduction in the development of lesions at the sites of wounds contaminated with *E. carotovora*. The term "tolerance to soft rot" should be amended, however, to read tolerance to the presence of *E. carotovora*, since the mere presence of viable cells of *E. carotovora* in a wound did not guarantee that a lesion would form (J. A. Bartz, *unpublished*). The formation of a soft rot lesion in a pepper or tomato fruit not only marked the end of palatability of that fruit, but also marked the point at which that fruit became a source of inocula for other fruit. The importance of tolerance to soft-rotting bacteria described herein and in the reports on tomato fruit (1, 2)

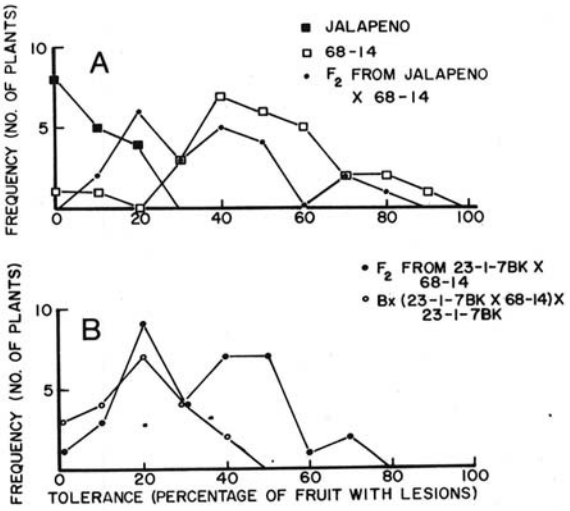

Fig. 1. Frequency of levels of tolerance of pepper fruit to *Erwinia carotovora* within plant populations from: **A)** Cultivar Jalapeno, 68-14, and F_2 from Jalapeno × 68-14; and **B)** F_2 from (23-1-7Bk) × (68-14), and backcross (23-1-7Bk × 68-14) × 23-1-7Bk.

thus involved: (i) reducing the number of fruit that could become centers of decay in boxes of fruit during transit, and (ii) actually increasing the number of fruit that could be consumed.

Fruit from Jalapeno peppers were more tolerant to *E. carotovora* than were those of other pepper lines tested. Stems and calyces of Jalapeno fruit were almost free of soft rot at the RH (ca. 95%) used in these tests. Though Jalapeno is a pungent pepper, tolerance to *E. carotovora* was not correlated with pungency. Fruit of Hot Portugal, Fresno Chili, Long Thin Cayenne, and Floral Gem, all pungent peppers, were just as susceptible to *E. carotovora* as the least tolerant sweet pepper. Neither was fruit shape correlated with tolerance. Yolo Wonder, a bell type, was significantly more tolerant to *E. carotovora* than were several other bell types such as Early Calwonder or Keystone Resistant Giant. One bell type, Jade, was even more susceptible than the latter cultivars. Four statistically distinct levels of tolerance among the test lines were computed. At least three levels of tolerance were found among the bell types alone.

To a considerable extent, 23-1-7Bk was derived from Yolo Wonder, and it seemed to possess the same level of tolerance as did Yolo Wonder. This suggestion, that tolerance could be inherited, was supported by data on the tolerance of parents and progeny in the test crosses. Two of the three levels of tolerance possessed by the parents in those crosses were recovered in the F_2 plants. But, the highest level of tolerance, that possessed by Jalapeno, was not recovered in the F_2. The intermediate level possessed by 23-1-7Bk was recovered in the F_2 plants from both crosses. In addition, the backcross (B×) of 23-1-7Bk with an F_1 (23-1-7Bk × 68-14) produced progeny with the same level of tolerance as did a selfing of 23-1-7Bk. This strongly implied that an intermediate level of tolerance could be achieved with only a short series of backcrosses.

The curve of the frequency of various levels of tolerance in the F_2 plants (Fig. 1) was not a normal curve, suggesting that tolerance was inherited by a relatively few number of independent genes, or groups of linked genes. The curves also implied at least three levels of tolerance. Those three levels correlated well with the four statistical levels of tolerance found among the different lines in Table 2.

The genetic data reported above were not based upon sufficient data to warrant definitive conclusions. Additional tests and test crosses will be needed before the exact inheritance of tolerance will be known. This report, however, did establish that tolerance to *E. carotovora* did exist in certain pepper lines and that such tolerance was inherited.

LITERATURE CITED

1. BARTZ, J. A., and J. P. CRILL. 1972. Tolerance of fruit of different tomato cultivars to soft rot. Phytopathology 62:1085-1088.
2. BARTZ, J. A., and J. P. CRILL. 1973. A study of methods for reducing bacterial soft rot in wounded fresh market tomatoes. Proc. Fla. State Hortic. Soc. 86:153-156.
3. GRAHAM, D. C. 1972. Identification of soft rot coliform bacteria, Pages 273-279 *in* H. P. Maas Geesteranus, ed. Proc. Third Int. Conf. on Plant Pathogenic Bacteria. Centre for Ag. Pub. and Doc., PUDOC, Wageningen, The Netherlands.
4. MC COLLOCH, L. P. 1962. Chilling injury and Alternaria rot of bell peppers. U. S. Dep. Agric. Mkt. Res. Rep. 536. 16 p.
5. STALL, W. M. 1973. An evaluation of fruit detachment characters in peppers (Capsicum annuum L.). Ph.D. Thesis, Univ. of Florida, Gainesville. 39 p.