

Variation in the Latent Period of Bacterial Soft Rot in Tomato Fruit

Jerry A. Bartz

Associate Professor, Department of Plant Pathology, University of Florida, Gainesville 32611.

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ABSTRACT

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The latent period (time between inoculation and water-soaking) of bacterial soft rot (BSR) in tomatoes wound inoculated with *Erwinia carotovora* subsp. *carotovora* (Ecc) ranged from less than 24 hr to more than 3 wk. This variation in latency was greater than could be accounted for by variation in the inoculation technique (needles previously dipped into inocula were thrust 2 mm into the flesh of a fruit). Wounds, inoculated with 5 μ l of bacterial suspension per 5 mm³ wound by using a micropipet, had more uniform numbers of cells of Ecc than did wounds from needles previously dipped in inoculum. By 24 hr after inoculation, however, the variance of numbers of bacteria in wounds in the two systems were equal to each other and were often significantly larger than their respective 0-hr values. The decrease in uniformity of the populations during the 24-hr incubation seemed to be associated with differences in the suitability of the damaged tissue for growth of Ecc. Concentrations of 10⁷ cells per milliliter led to populations in wounds 10-fold greater than in wounds inoculated with 10⁶ cells per milliliter, not only immediately after inoculation, but also

at 18, 24, and 36 hr. When the number of cells approached 10⁷ per wound, growth of Ecc appeared restricted. Tenfold differences in the inoculum concentration (10⁹ through 10⁵ cells per milliliter) led to approximately twofold differences in the incidence of BSR at 48 hr. Disease progress curves were of the exponential or restricted growth shape whether the percentage of fruit with lesions after inoculation or the percentage of diseased wounds on individual fruit was plotted. The curves approached asymptotes whose levels varied according to the levels of initial disease; higher inoculum dosages generally led to higher levels of initial disease and higher asymptotes. Disease progress after inoculation with *Pseudomonas marginalis* was similar. Because most inoculated fruit finally succumbed to BSR during prolonged storage, the asymptotes not equal to 100% disease were interpreted as evidence for a host reaction that temporarily localized the bacterium. The temporary nature of the host reaction seemed responsible for the variable latency.

Additional key words: *Lycopersicon esculentum*, postharvest decay.

The incidence of bacterial soft rot (BSR) after wound inoculation has been used to measure the effects of fruit ripeness, chilling, certain fertilization practices, and cultivar on the susceptibility of tomato fruit to *Erwinia carotovora* subsp. *carotovora* (Jones, 1901) Bergey et al, 1923 (Ecc) (1-4). Susceptibility was defined as the incidence of BSR (numbers of diseased fruits) at given postinoculation intervals rather than on the rate of lesion expansion (2). The onset of symptoms, however, was seldom uniform either on individual fruits or treatment replicates. Frequently, extensive variation in the incidence of BSR among replicates obscured differences among treatments.

The extensive variation in BSR incidence and, especially, the delay in disease occurrence at some wounds were investigated to determine if tomatoes temporarily resisted infection by the pathogen. In the following report, the variation in the latent period of BSR was illustrated and proved to be a characteristic of the disease, not the result of inoculation techniques.

MATERIALS AND METHODS

Fruit. Healthy, hand-harvested, Florida-grown tomatoes were inoculated when fruit color was green to red. All fruit within the same treatment replicate were of the same cultivar and of the same color. The wounding of unwashed fruit with sterile needles seldom resulted in BSR lesions, indicating that fruit used in the tests were not only free of incipient BSR lesions but also free from extensive contamination by Ecc.

Inoculum. Stock suspensions of a Florida isolate of Ecc, SR-12 (2), were grown in nutrient broth as shake-cultures for about 24 hr at approximately 26 C. The bacteria were pelleted by low-speed centrifugation, resuspended in a dilute saline solution buffered to pH 7.0 (0.8 g NaCl, 0.2 g KH₂PO₄, and 0.2 g Na₂PO₄ in 1 L of glass distilled water), and diluted to a turbidity of 0.1 to 0.8 optical density (OD) at 600 nm. Suspensions of Ecc at an OD of 0.3

contained $\sim 2 \times 10^8$ cells per milliliter. Appropriate dilutions were made as needed for the various tests. An isolate of *Pseudomonas marginalis* (Brown, 1918) Stevens, 1925 (Pm) (isolated from a tomato fruit in Florida) was handled similarly.

Inoculation techniques. *Wound inoculation.* A cork pierced by four straight pins was mounted on a holder. The pin points, which protruded by ~ 2 mm, and the face of the cork were dipped into a suspension of bacteria and then thrust into the sides of a fruit, making a wound site composed of four holes (2-3 mm apart) of about 1.6 mm³ volume each (1,2). In some tests, the wound site was a single pinhole made by one pin per cork. This process was repeated with each fruit as often as needed to provide the required number of wound sites, each at least 2 cm apart.

Wound-dip inoculation. A wound-inoculation instrument described above was sterilized and then used to make a predetermined number of wounds after which the fruit were immersed for 1 min in a suspension of Ecc, then removed and allowed to air-dry before being placed in storage.

Micropipet inoculation. The surface of the fruit was wounded to a depth of 2-3 mm with a 1.5-mm-diameter blunt probe. Five microliters of a suspension of Ecc were pipetted into each wound whose volume was ~ 5 mm³.

Storage and observation. Inoculated fruits were stored under high humidity in a Model Mini-48 controlled environment cabinet (Scientific Systems, Inc., Baton Rouge, LA 70815). The temperature was regulated from 20 to 30 C for the different tests, but was constant, ± 0.5 C, within each test. The relative humidity in the chamber was measured electronically at 75-80%. Inoculated fruits were observed at 2- to 48-hr intervals. Diseased fruits were discarded within 48 hr after the onset of symptoms in order to prevent secondary spread of the bacteria (2). The first observation was timed to occur after at least 5% of the fruits were diseased, but before the first lesions were more than 48 hr old.

Counts of viable bacteria. Numbers of Ecc cells in wounds were estimated with a dilution-plate technique. Fruits were wiped before inoculation with a paper towel soaked in a 10% solution of laundry bleach, 5.25% NaClO. At designated intervals after inoculation, a

wound site was aseptically excised with a 9-mm-diameter (No. 6) cork borer and excess tissue was removed with a flamed scalpel. The remainder was placed in a tube containing sterile buffered saline and crushed with a sterile glass rod. Dilutions were made as needed with a microtiter instrument (Microtiter, Cooke Engineering Co., Alexandria, VA 22314). Sample volumes, 0.05 ml, from the last two or three dilutions were spotted on half of a crystal violet-polypectate medium plate (6). The spots were spread uniformly over each half-plate with a flamed, bent glass spreader. The plates were incubated at 30 C. Depressions in the medium that developed within 24 hr were counted as colonies of Ecc. Detectable Ecc (at least 30 cells) were not found on the dried surface of unwounded tissue either initially or 24 hr after that tissue had been touched with a cork wet with inoculum.

Statistical analysis. Numbers of bacteria were converted to \log_{10} values as suggested by Steel and Torrie (13). Variances (s^2) of the estimated populations for different treatments were compared by Bartlett's test for the homogeneity of variances. The significance of the differences between the means of two treatments was determined by the one-way analysis of variance, that between two variances by the *F*-test. Potential correlations were tested by the linear regression, correlation coefficient test.

Experiments. Variation in the latent period. Data from nine different tests on the resistance of BSR in tomatoes were reported as: the percentage of fruit with BSR at the first observation after inoculation, the percentage of diseased fruit with lesions at more than one wound site, and the percentage of diseased fruit with lesions at all four wound sites. The number of fruit inoculated in a test ranged from 270 to 530. Each fruit was wound inoculated in four locations (four holes per site) with suspensions containing 5×10^6 cells of Ecc per milliliter as described above. In a subsequent test, each of 329 fruits of one cultivar and two breeding lines were inoculated with a series of 10-fold dilutions (one puncture per dilution), 10^9 through 10^5 cells per milliliter. The fruits were harvested at the mature green stage of ripeness, and kept at 20 C; sublots of pink fruit were removed and inoculated on three separate dates. The inoculated fruits were observed 18, 24, and 48 hr after inoculation.

Variation in the number of cells of Ecc in wounds. Variation in bacterial number inherent in the dilution-plate technique was calculated from single plates prepared from separate dilutions of 10^7 cells per milliliter of suspension contained in each of three separate Erlenmeyer flasks. The three suspensions were prepared from a single stock suspension. A total of 12 different stock suspensions were prepared, each from a different nutrient broth shake culture, on different dates.

The number of cells of Ecc in symptom-free wounds on susceptible fruit was determined. To assure that the symptom-free sites were in a state of prolonged latency with regard to BSR, diseased fruits with at least one symptom-free wound site were selected from a group of fruit 3 days after inoculation; the lesions were excised and the fruits were returned to storage at 20 C. After 3 days, the numbers of Ecc in 11 four-puncture wound sites that remained symptomless were counted as described above.

To estimate the uniformity of the populations of Ecc deposited in wounds by three different inoculation techniques, inoculated fruits were sampled immediately after inoculation (0 hr), then stored at 26 C for 24 hr and sampled again. Sample variances, s^2 , were the parameters of variability in the sizes of the different populations. The relative size of a 24-hr population in a single puncture inoculated by wound inoculation was compared by linear regression, correlation coefficient analysis to the percentage of the remaining punctures that were then diseased or were diseased when observed 24 hr later. This was done once with 30 pink fruits, each wounded with 20 punctures, and twice with green fruit similarly wounded.

The colonization of four-puncture wound sites by Ecc was monitored by sampling such sites at 0, 18, 24, and 36 hr. The mature green fruits were wound inoculated with 10^8 , 10^7 , and 10^6 cells per milliliter suspensions, respectively, and were stored at 26 C.

Disease-progress curves. Disease progress, the percentage of fruit

with BSR, among the 329 pink fruits inoculated in the latent period experiment was plotted over time for the 10^9 and 10^8 cells per milliliter inoculum dosages. Subsequently, disease progress curves were drawn for Ecc and Pm in 10 red tomatoes, each with 30 wounds. Three concentrations of Ecc were used, 10^9 , 10^8 , and 10^7 cells per milliliter, with 10 punctures on each fruit from each dosage. A second group of fruit was similarly inoculated with suspensions of Pm. Disease progress was the percentage of wounds in which lesions occurred over time. The fruit were held at 26 C and observed periodically until existing lesions expanded into adjacent, symptomless wound sites.

Terminology. The latent period is the time interval between the introduction of inocula into wounds and the production of secondary inocula. The latter event coincides with the onset of tissue maceration, denoted by water-soaking, because at that time Ecc can spread by contact to new hosts. This usage of the term, latent period, is similar to that of Zadoks and Schein (15).

RESULTS

Variation in the latent period of BSR. Examples of variability in the incidence of BSR were evident in the results of the nine tests selected to illustrate variable latency (Table 1). First, the disease appeared at different intervals after inoculation with a standard inoculum concentration, 5×10^6 cells per milliliter, and a standard storage environment, 20 C and $\geq 75\%$ relative humidity. This could be explained by differences in susceptibility of the fruit but what could not be explained was that from 3 to 39% of the fruit in those tests were still disease-free 10–14 days after the initial observation. These healthy fruits were not simply members of the most resistant treatments or cultivars. Second, the appearance of BSR at the wound sites on a given fruit was not uniform; the uniformity was not associated with the time or the incidence of the initial disease. When the diseased fruits were discarded, an average of 42% had a lesion at only one wound site. Conversely, only 9% had lesions at all four wound sites. The percentage of diseased fruit with multiple lesions was positively correlated with the initial incidence among the treatments on only five of the nine tests. Thus, a high incidence of BSR at the initial observation was only partially associated with the tendency for multiple lesions. Moreover, diseased fruits in the test with the highest incidence at the earliest date, 61% at 2 days, were not more prone to have multiple lesions than were those in the other tests. Rather, they were the second lowest in terms of the percentage of diseased fruit with multiple lesions.

In a subsequent test, variation in the appearance of BSR over time occurred among wounds inoculated with a series of 10-fold dilutions of a suspension of Ecc. The percentage of wounds that were diseased decreased by about twofold as the inoculum concentration was decreased by 10-fold. At 18 hr after inoculation, 13, 1, 0, 0, and 0% of the wounds inoculated with the 10^9 through 10^5 cell suspensions were diseased, respectively. By 48 hr, those disease incidences had increased to 56, 20, 9, 2, and 1%, respectively. The incidence of disease varied among the three inoculation dates. By 48 hr after inoculation, 96% of the fruit in the first test had BSR, whereas in the second and third tests, 83 and 58%, respectively, were diseased. Variation in the appearance of BSR on individual fruit was also noted. At 18 hr, four of 79 diseased fruits had symptomless wound sites that had been inoculated with the most concentrated inoculum; by 48 hr, corresponding values were 20 of 195 diseased fruits. Failure of BSR to appear at sites receiving more Ecc than nearby diseased sites occurred on five diseased fruits at 18 hr and on 55 at 48 hr. At the latter observation, 12 fruits had symptomless sites that had been inoculated with at least a 100-fold more concentrated suspension than had nearby diseased sites.

Variation in the level of disease resulting from the three separate but similar inoculations of fruit from the same lot may have been caused by different inoculum concentrations. The average viable cell count for the 12 stock suspensions whose concentration was estimated by OD, then diluted to 10^7 cells per milliliter was 4.8×10^7 cells per milliliter with a range of 10^7 to 9.6×10^7 and a standard deviation of 2.6×10^7 . Up to 10-fold differences in numbers of

bacteria might occur in different inoculum preparations, although smaller differences would be more likely. However, this did not explain the presence of symptomless wounds on diseased fruit.

Variation in the numbers of cells of Ecc in wounds. One of many possible explanations for fruit with symptomless wounds that were inoculated with more bacteria than nearby diseased wounds was that in those sites the bacteria either did not survive introduction into the wounds, did not multiply, or after multiplying began to die. However, viable Ecc were found in symptomless wound sites 3 days after BSR appeared at adjacent wounds. The populations appeared relatively uniform in size and high in number. The average population from 11 different samples was 2.4×10^7 cells. The range was 1.3×10^7 to 3.7×10^7 cells. These values were in accord with previous observations (2, and unpublished). The symptomless wounds did not result from a failure of the Ecc to survive at relatively high concentrations. Furthermore, in subsequent tests, viable Ecc were always detected in inoculated wounds and always multiplied when inoculated fruits were stored.

Previously, the size of the populations in wounds 24 hr after inoculation was associated with differences in lesion incidence at later time intervals (2). The possibility that the inoculation technique resulted in the deposition of different-sized populations among the wounds and that these differences in population size affected at the time of lesion appearance was examined. Variation in population size among wounds was estimated with sample variances, s^2 . \log_{10} transformed numbers were used so that differences among treatment values by 10^n would not bias the comparisons. Among the transformed viable cell counts of the 12 different inoculum preparations, the average s^2 was 0.0062. This was similar to the average, 0.0069, for the 12 micropipet inoculations in which exactly 5 μ l of inoculum was introduced into a 5-mm³ puncture. Thus, the variation inherent in diluting inocula and spreading the final dilution on a plate was not increased by adding inoculum to a wound and then sampling and crushing that wound to liberate the bacteria. This variation was much smaller than that of the viable cell count for the 12 different stock suspensions, $s^2 = 0.076$.

The average s^2 , 0.046, of the populations of Ecc deposited in the various punctures by the wound-inoculation technique was higher ($P = 0.005$) than that resulting from the micropipet technique (Table 2). However, after 24 hr, the s^2 in the two techniques were similar. In seven of the 12 micropipet inoculations and one of the three wound inoculations, the 24-hr populations were more

TABLE 1. Variation among nine different tests in the appearance of bacterial soft rot in tomato fruit 2-4 days after inoculation^a with *Erwinia carotovora* subsp. *carotovora* (Ecc)^b

Fruit/test (no.)	Disease ^c at days after inoculation			Fruit with:		Correlation ^d (r) of disease with multiple lesions
	2 days	3 days	4 days	Multiple diseased wound sites	Four diseased wound sites	
450	-	11	+	55	6	NS
450	30	+	+	89	21	0.96 **
530	-	13	+	55	5	0.75 **
270	-	16	+	26	2	NS
270	61	+	+	52	7	0.63 **
270	11	+	+	52	0	NS
270	-	32	+	53	13	0.45 *
270	32	+	+	65	17	NS
360	-	-	33	60	6	0.52 *

^aFour straight pins mounted on a cork were dipped into a suspension of 5×10^6 cells Ecc per milliliter of buffered saline and were then thrust onto the sides of the fruit. This was done at four sites on each fruit and then the fruit were stored at 20 C and high humidity.

^bFlorida isolate SR-12.

^c- = 0-4% of fruit with lesions, no recorded observation; + = \geq value on preceding day.

^dPercent fruit with at least one lesion at first recorded observation vs percent fruit with multiple lesions within each test. NS = No significant correlation by Student's *t*-test; * = significant at $P = 0.05$; ** = significant at $P = 0.005$.

variable ($P \leq 0.05$) than were those at 0 hr. Among the various inoculum concentration treatments in the micropipet inoculation, the highest cell count was 1.1-2.3 times the lowest at 0 hrs, but 1.4-5.3 times at 24 hr. Similarly, in the wound-inoculation treatments, the ratio of the highest to lowest cell count were 2.0 to 5.6 at 0 hr, but 21 to 325 at 24 hr. Finally, the s^2 at 24 hr was not correlated with that of the 0-hr populations. Variation in numbers of bacteria deposited per wound with the wound-inoculation system did not appear responsible for variable numbers of generations of Ecc during the first 24 hr after inoculation, because considerable variation also occurred in the micropipet system. On the other hand, that variation could be caused by the host. The average populations in the larger wounds in red fruit were up to 10-fold greater at 24 hr than those in green fruit. Fruit ripeness affected the multiplication of Ecc; other fruit characteristics such as inherent susceptibility might do the same.

Additional green fruits were wounded and then immersed in a suspension of Ecc. The average variances of the initial and 24-hr populations of Ecc, 0.072 and 0.264, respectively, in these tests were not different from those resulting from wound inoculation. However, the inoculum concentration for the immersion technique had to be six times greater than that used in the wound-inoculation technique in order for each puncture to receive the same number of

TABLE 2. Average number of cells^a of *Erwinia carotovora* subsp. *carotovora*^b (Ecc) in wounds in tomato fruit at 0 and 24 hr after inoculation by two different methods^{c,d}

Inoculation method	Fruit color	Inoc. conc.	Population mean		Variance ^e (s^2)		F ₂ -test ^f (s^2_0 vs s^2_{24})
			0-hr (10^2)	24-hr (10^5)	0-hr	24-hr	
Micropipet	Green	10^8	5,900	3,900	0.004	0.021	5.76
		10^7	640	2,300	0.005	0.470	90.35 *
		10^6	66	250	0.001	0.143	158.67 **
Micropipet	Red-1	10^8	6,000	44,000	0.005	0.547	113.85 ***
		10^7	550	38,000	0.023	0.159	6.82
		10^6	59	440	0.015	0.020	1.37
Micropipet	Red-2	10^8	5,400	81,000	0.005	0.152	29.84 ***
		10^7	820	20,000	0.002	0.304	144.95 ***
		10^6	89	930	0.002	0.004	2.93
Wound inoculation	Green-1	10^7	18	2.8	0.008	0.112	14.36 ***
		Red	84	11	0.042	0.240	4.58
		Green-2	6	12	0.087	0.162	1.86

^aAverage of five wounds except for micropipet at 0 hr (three wounds), green pin-punctures (three wounds) and all 24-hr pin-punctures (30 wounds).

^bFlorida isolate SR-12.

^cMicropipet—5 μ l of 10^8 , 10^7 , 10^6 , or 10^5 cells suspension of Ecc per milliliter were pipetted, respectively, into a 2-mm-deep, 2-mm-diameter hole in the wall of a fruit. The inoculated fruits were stored at 20-22 C.

^dWound inoculation—a straight pin was dipped into a 10^7 cells/ml suspension of Ecc then thrust to a depth of 2 mm into the wall of the fruit. The inoculated fruits were stored at 25-26 C.

^eVariance (s^2) \log_{10} -transformed numbers of cells.

^fTest of significance for difference of variance at 0 hr from variance at 24 hr; * = significant at $P = 0.05$, ** at $P = 0.025$, *** at $P = 0.01$.

TABLE 3. Average percentage of inoculated sites that were diseased 48 hr after immersion of tomato fruit with pin-puncture wounds 0, 4, and 16 hr old for 30 sec into suspensions containing 10^7 and 10^8 cells of *Erwinia carotovora* subsp. *carotovora* per milliliter of distilled water^{a,b}

Age (hr)	Inoculum concentration	
	10^8	10^7
0	25	5
4	25	10
16	50	10

^aAverage of two 10-fruit duplicates.

^bStored at 26 C and >75% relative humidity.

bacteria. Wounds at least 2 hr old absorbed four to eight times more Ecc than did fresh wounds. The means of the respective populations in old vs fresh wounds were similar at 24 hr. Older wounds appeared less favorable for multiplication of Ecc than did fresh wounds.

The difference between old and fresh wounds with regard to initial populations did not result in different levels of disease (Table 3). Wounds 16 hr old when immersed, developed BSR just as rapidly as those that were 0 or 4 hr old. Apparently, within limits (up to eightfold differences), the numbers of Ecc initially introduced into wounds were not as important in determining the formation of a lesion as were the numbers present at 24 hr. However, in three separate tests with 30 fruits in each test, the 24-hr population in a single wound could not be correlated with the frequency of disease among the remaining 19 wounds, $r = 0.10$, -0.14 , and -0.09 , respectively. In test 2, with the red fruit, 73% of the fruit were diseased at 24 hr; the largest population found was $\sim 6.5 \times 10^6$ cells and the percentage of wounds diseased on each fruit ranged from 0 to 63. In the two green-fruit tests, lesions were not found at 24 hr, but were present at 48 hr; the largest population recorded at 24 hr was 7.1×10^6 cells. In one test, the percentage of wounds diseased on each fruit ranged from 0 to 79, whereas in the other, range was from 16 to 100%.

Assuming that at least one of the 90 different wounds sampled in the preceding test was of an incipient lesion, then the critical population density number associated with visible tissue maceration would be $\sim 7 \times 10^6$ cells per 1.6-mm³ puncture. This value compares with that reported for potato tissue by Perombelon (12), 10^6 cells per lesion. The symptomless four-puncture wounds sampled from diseased fruit earlier contained critical densities of Ecc but remained symptom-free. The increase in numbers of Ecc cells in wounds in tomato fruit need not terminate with the onset of tissue maceration. On the contrary, active multiplication often ceased before symptoms occurred. This fact diminished the importance of the numbers of bacteria introduced into wounds on the appearance of lesions over time.

The rate of colonization of four-puncture wound sites by Ecc was not affected by the size of the bacterial populations introduced into the wounds. The numbers of Ecc in the wound sites at 0 hr were correlated with inoculum dosages from 10^6 to 10^8 cells/ml (Table 4). Populations in wounds inoculated with the 10^7 dose were 10-fold greater than those in wounds with the 10^6 dose at 18, 24, and 36 hr after inoculation. The numbers of Ecc in wounds in the 10^8 treatment did not increase significantly between 18 and 24 hr. Multiplication of Ecc slowed as the critical density was approached.

The numbers of cells in wounds in the 10^6 and 10^7 treatments increased by about 100-fold (seven generations) during the first 18 hr, but only 10-fold (three generations) during the second 18 hr. This apparent reduction in the multiplication rate was not associated with the populations reaching critical density since the larger population at 36 hr was only approximately 10% of the critical density or just at a point where a reduction in the multiplication rate could be expected. The reduction of the multiplication rate after 24 hr, regardless of the number of bacteria present, was evidence that changes in the damaged host tissues

restricted growth of Ecc.

Disease-progress curves. In the first tests reported here, BSR resulting from a single inoculation date appeared over an extended time period that ranged from 18 hr to >3 wk. Fewer new lesions appeared during later time intervals. Plots of the increase in percentage of fruit with BSR after a single inoculation with suspensions of 10^9 and 10^8 cells per milliliter over time resembled a variation of exponential curves sensu Zadoks and Schein (15) (Fig. 1). This result was expected for simple interest diseases (14,15). The curves approached an asymptotic level during the interval from 36 to 48 hr after inoculation. Curves that originated at different initial disease levels, X_0 , had different asymptotic levels that were considerably less than $X = 1.0$. The X_0 s were not always dependent upon the inoculum concentrations either between or within tests, although inoculations with the 10^8 suspensions had consistently lower X_0 s and lower asymptotic levels. Differences in the X_0 between tests might be explained by different viable cell counts in the inoculum concentrations as suggested earlier.

Similar curves resulted when 10 fruits were each inoculated several times with three dosages of Ecc or with three dosages of Pm (Fig. 2). Exponential curves with an asymptote varying according to X_0 were also characteristic of BSR caused by Pm. With Pm, lesion expansion was not as rapid as with Ecc. Consequently, the fruit in the Pm tests were observed an additional 80 hr after those in the Ecc test had to be discarded. Only 10 of 241 symptomless wounds at 48 hr became diseased in the next 60 hr of storage and only one new lesion appeared in the last 36 hr. This reinforces the conclusion that the asymptotes need not equal 100% disease.

DISCUSSION

Bacterial soft rot has an extremely variable latent phase in tomato fruit. Disease appeared on some fruit within 2 days after wound inoculation; however, others of the same lot and of the same ripeness treated identically were still free of disease 14 or more days later. This might be explained by heterogeneity in resistance to BSR except that variable latency may be demonstrated among wound sites on individual fruit. The causal bacterium was always isolated from inoculated wounds; it always multiplied during storage of the inoculated fruit. Thus, the variation was not due to failure of the pathogen to enter or survive in the wounds.

A more plausible explanation and one more difficult to disprove was that variable latency was caused by variation inherent in the wound-inoculation technique especially with regard to the number of bacteria deposited in each wound. However, there are three different observations that are inconsistent with this argument. First, populations of Ecc in wounds in tomatoes increased rapidly to near-critical densities, and if a lesion did not form, the bacteria remained quiescent for an indefinite period of time. This maybe contrasted with anaerobically incubated potato tuber slices in which tissue maceration is visible when Ecc multiplies to critical density (12). With *P. phaseolicola* in bean leaves and *P. morsprunorum* in cherry leaves, transition of bacterial growth from log to stationary phases was accompanied with symptom onset (7). With *E. chrysanthemi* in pineapple, the pathogen causes a limited water-soaked necrosis at the base of the styler canal within a fruitlet, then remains quiescent for 20 mo during fruit development (10). Shortly before ripening of the infected fruit, the organism resumes multiplication, causing a rapid disintegration of the host.

Second, the variation inherent in the wound-inoculation technique was not sufficient to account for the large variation in population size at 24 hr after inoculation or for the variable incidences of BSR. With *P. phaseolicola* and *P. morsprunorum*, the generation time of these bacteria in their homologous hosts was not greatly affected by the inoculum dose; however, growth ceased earlier with higher doses (7). As noted herein, the growth of Ecc in tomatoes also ceased earlier at higher dosages. However, the multiplication rate of Ecc was reduced at ~ 24 hr after inoculation, regardless of the size of the population, evidence that multiplication of Ecc in wounds was affected by the host. Furthermore, an initial 5.6-fold difference in the size of the populations in wounds on various fruit immediately after

TABLE 4. Numbers of cells of *Erwinia carotovora* subsp. *carotovora* (Ecc) in wound-inoculated tomato fruit at various intervals after inoculation with three inoculum concentrations^{a,b}

Inoculum concentrations (cells/ml)	0 hr (10 ²)	18 hr (10 ⁴)	24 hr (10 ⁵)	36 hr (10 ⁵)
10 ⁸	4	6	2	5
10 ⁷	110	88	12	53
10 ⁶	930	430	58	69

^a Average number of pectolytic bacteria from three crystal violet polypectate plates streaked with a diluted bacterial suspension prepared from the crushed tissue of one four-puncture wound site.

^b Each fruit was wounded four times with four straight pins that had been dipped into a suspension of Ecc.

inoculation increased to 325-fold within 24 hr. Clearly, during the first 24 hr after inoculation, the increase in the population of Ecc varied from wound to wound on fruit from the same lot. Some wounds appeared more suitable for colonization by Ecc than others. The failure of the population size in a single wound on a fruit to be correlated with the percentage of the remaining 19 wounds that are diseased 24 hr later is suggestive that variation in population size at 24 hr among wounds on a fruit would be similar to that demonstrated among fruit.

Third, more uniform initial populations did not lead to more uniform 24-hr populations. Pipetting 5 μ l of inoculum into a 5-mm³ hole in the wall of a fruit led to very uniform initial populations but the average sample variance of the 24-hr population with this technique was similar to that with the wound-inoculation technique. The larger wounds simply led to more bacterial growth, ~ 14 vs eight generations in 24 hr. Thus, within limits, variable populations in wounds at 24 hr after inoculation and, ostensibly, variable latency were independent of variation in the 0-hr populations. Indeed, the variance at 0 hr in the micropipet tests was not correlated with that at 24 hr. The upper limits of variability for the initial population before a variation in BSR incidence in tomatoes could be expected to be larger than that inherent in the wound-inoculation system. Thus, based on the three observations discussed above, the variable latency described herein could not have been caused by inconsistencies in the wound-inoculation technique.

Disease progress for BSR followed restricted growth curves, referred to as exponential by Zadoks and Schein (15). The form was that of a typical simple interest disease (14,15). This was expected; secondary spread (polycyclic cycles) was discouraged if not

prevented. Disease progress after inoculation with Pm was similar to that in fruit inoculated with Ecc. This provides additional evidence that the host and not the pathogen was responsible for the slowing in the rate of lesion appearance over time. Interestingly, with both Ecc and Pm, asymptotes appeared at percentages of diseases which depended on the initial amount of disease. In a previous report, inoculated fruit were held until all fruit had BSR (2); a prolonged storage period, over 4 wk, was required. This result is difficult to reconcile with the apparent asymptote response unless the progress of BSR from ~72 hr on follows a second curve with a shape and slope different from those of the first.

The asymptote response could be explained by a host resistance reaction that was completed in the host by ~48 hr. Lovrekovich and Stahmann (11) reported that the potato tuber's polyphenol oxidase system could stop the progress of tissue maceration caused by the pathogen's pectic enzymes. Fox (8) suggested that under aerobic conditions, Ecc was localized by the host's suberization of tissue surrounding wounds or natural openings (lenticels). Perombelon (12) reported that rotting of potato tuber disks in the presence of O₂ depended on whether the Ecc or the closely related *E. carotovora* subsp. *atroseptica* (van Hall, 1902) Dye, 1969, could multiply fast enough to reach critical population densities before suberization of the tissues restricted their growth. Hall et al (9) reported a polyphenol oxidase system that is activated by bacterial activity in tomato fruit. Lim and Lowings (10) concluded that a high polyphenol oxidase activity was responsible for the localization and quiescence of *E. chrysanthemi* in developing pineapple fruit. Polyphenol oxidase or suberization activities are not as vigorous in tomatoes as in potato. Cut surfaces of tomatoes

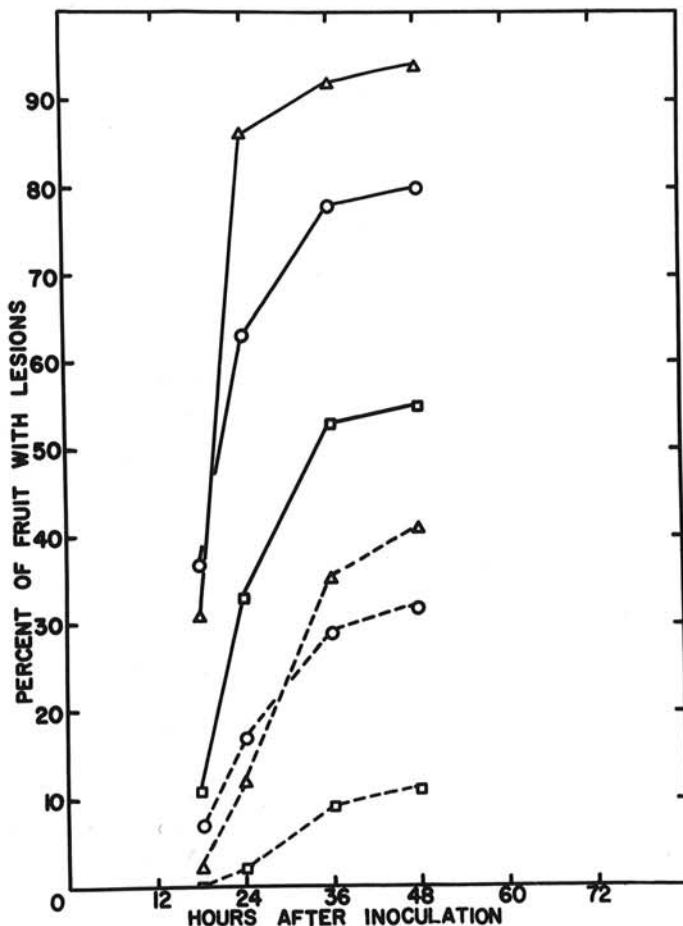


Fig. 1. The percentage of tomato fruit with lesions at various time intervals after wound inoculation with a single pin that had been dipped into a 10⁹ (—○—) or 10⁸ (—Δ—) or 10⁷ (—□—) cells of *Erwinia carotovora* subsp. *carotovora* per milliliter buffered (pH = 7.0) saline solution. The test was performed three times (O = first test; Δ = second test; and □ = third test).

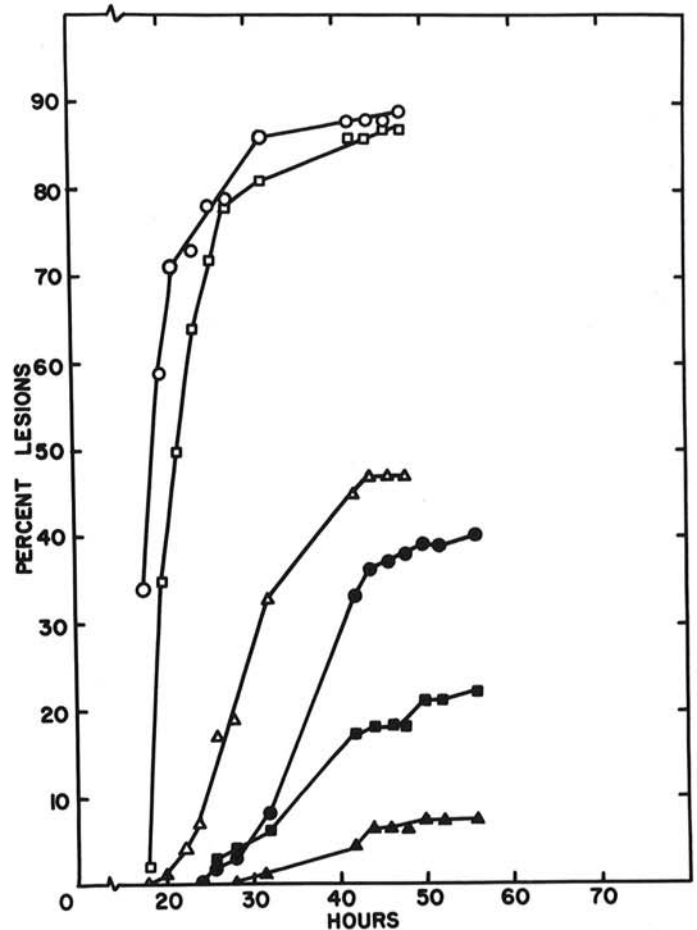


Fig. 2. The average percentage of wounds that have become diseased at various time intervals after wound inoculation of 10 tomato fruit 10 times per fruit with a single pin that had been dipped into a suspension containing 10⁹ (O + ●), 10⁸ (□ + ■), or 10⁷ (Δ + ▲) cells of *Erwinia carotovora* subsp. *carotovora* (open symbols) or *Pseudomonas marginalis* (closed symbols) per milliliter.

exposed to air do not turn brown nor does extensive corking follow wounding. However, the variable latency phenomenon as well as the asymptote response could be explained if the host reaction at least temporarily localized the pathogen. In any case, variable latency is a characteristic of bacterial soft rot in tomatoes whether inoculated by wound inoculation or by infiltration (5, and unpublished). It is not an artifact caused by a particular method of inoculation.

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