Bacterial Vascular Necrosis and Rot of Sugarbeet: Effect of Moisture, Age of Plants, Injury, Inoculation and Genotype on Susceptibility to Infection by Erwinia

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INTRODUCTION

Bacterial vascular necrosis and rot in sugarbeet (*Beta vulgaris* L.) was discovered in 1972 (6). An understanding of environmental factors that contribute to disease is important for developing measures to reduce its severity. Field observations suggested that moisture conditions (type of irrigation or low-lying spots in the field), age of plants at the time of infection, injury, and genotype may have some effect on the susceptibility of plants to the bacterium. Therefore, greenhouse and field studies were conducted to determine the effect of moisture on the susceptibility of sugarbeet to *Erwinia caratovora betavasculorum*, and the effect of plant age, injury and genotype at the time of inoculation on susceptibility.

MATERIALS AND METHODS

Greenhouse tests. Six-week-old sugarbeet plants were injured by piercing four petioles (1 cm from the base) of each plant with a dissecting needle. Cultivars known to be resistant, intermediately susceptible, or susceptible to the pathogen were used (Table 2). Twelve and 18 plants, Test One and Two, respectively, of each cultivar were placed either on greenhouse benches or in a chamber in the same greenhouse where the plants were misted intermittently with water (5 seconds every 2½ min.). Plants were inoculated 0, 1, 2, 4 and 8 hrs. after injury with a suspension of 10^7 cells per ml of the sugarbeet *Erwinia* isolate SB 13. The inoculum was prepared as previously described (8). About 0.25 ml was atomized onto each injured petiole. Each plant receiving the mist treatment

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was placed immediately after inoculation beside one inoculated with the sugarbeet *Erwinia* but not misted. Plants were arranged in a randomized complete block design. The number of infected petioles was recorded 1 week after inoculation. Temperatures in the greenhouse were maintained between 26 and 35°C.

Field studies. Irrigation experiments were designed as split-split plots with furrow vs sprinkler irrigation treatments as main plots (4 replications), inoculations as subplots, and cultivars as sub-sub plots. Buffer zones 3-m wide with plants were retained between irrigation treatments and sprinkler irrigation was applied only during non-windy periods to avoid the drifting of water to furrow irrigated plots. The cultivars used were US H10, US H7A, C17, US75 546H3, Maris Vanguard and Y03 in 1973 (two tests); cultivars US H10, US H7A, C17 and 546H3 in 1974 (one test). The plants were thinned to a 15 to 20 cm in-row spacing when 4 weeks old and inoculated 6 weeks later as previously described (8).

The effect of plant age on susceptibility was determined in randomized complete block designed experiments at Salinas and Spence, CA in 1975. Each age was replicated six times. Two cultivars, susceptible C17 and resistant C64 were used. Seed was sown at 2 week intervals in May and June, thinned to 15 to 20 cm in-row spacing, plants were inoculated once when the plants were 6, 8 and 10 weeks old.

Five months after planting the beets of the irrigation tests and age of plant tests were harvested, weighed, evaluated for rot (disease index, DI=% rot per beet/number of beets per plot) (8) and analyzed for sucrose percentage. The visually-estimated rot increments were 0, 7, 25, 50, 75, 93 and 100 (2). The data from the age of plant tests at Salinas and Spence, CA were homogeneous, so were combined for the analysis.

Injury x cultivar x inoculation studies were designed as factorial experiments with *Erwinia* inoculations as whole plots, injury as subplots and cultivars as sub-sub plots. Each sub-sub plot was replicated four times per test per year. Eight cultivars were used, ranging from resistant to susceptible (Figure 4). Four California isolates of Erwinia (SB-4, SB-6, UR-7 and SB-13) were used in equal amounts for the inoculum. Individual plants were injured by crushing the leaves, and petioles with a doughnut-shaped metal plate attached to a handle resembling a ski pole. Appropriate treatments were applied when plants were 10 weeks old. The plots were planted in late April or early May at Salinas and Spence, California in 1976 and 1977 and harvested when plants were 6 months old. Each root was sliced at harvest time to estimate the percentage rot per beet. Root yield also was determined and DIs were calculated as described above.

RESULTS

Greenhouse studies. Misting of injured petioles increased the length of time that the injuries remained susceptible to infection (Table 1). Resistant and very susceptible cultivars were affected less by misting than were intermediately susceptible cultivars (Table 2).

Table 1. Percentage of infected sugar beet petioles 1 week after inoculation with *Erwinia* sp. at different times after petiole injury.

	Hours between injury and inoculation						LSD[a]
	0	1	2	4	8	$\bar{x}$	0.05
Test 1[b]							
Misted	45.8	33.3	33.3	31.3	22.9	33.9	
Nomisted	37.5	22.9	16.7	10.4	6.3	18.8	5.3
Test 2[b]							
Misted	79.2	40.6	41.7	25.0	12.5	39.8	
Nomisted	72.9	40.6	14.6	14.6	2.4	29.0	10.4

[a]To test differences between means of misted vs nonmisted within each test at each hr between injury and inoculation.
[b]Test 1 had 48 reps and test 2 had 96 reps.

Field studies. Sprinkler irrigation significantly increased the DI and percentage infected beets when analyzed over years, 1973-1974 compared to furrow irrigation (Table 3). Sprinkler irrigation had no effect on percentage rot per infected beet, yield or percentage sucrose.

Table 2. Effect of leaf wetness and injury on the susceptibility of sugar beet cultivars differing in resistance to *Erwinia* sp.

		% infected petioles					
	Host	Test 1[a]		Test 2[b]		Total	
Cultivar	response	Non-misted	Misted	Non-misted	Misted	Non-misted	Misted
C17	S[b]	38.9	41.7	29.2	37.5	35.00	40.0
Y04	I	22.2	45.8			22.2	45.8
US 75	I	11.1	27.8	22.9	31.3	15.8	29.2
554H1	I	12.5	29.2	12.5	37.5	12.5	24.2
Y03	R			10.4	8.3	10.4	8.3
SP7035	R			16.7	14.6	16.7	14.6
x		21.2	36.1	18.3	25.8	19.6	30.4

[a]Each value based on 48 petiole inoculations in test 1, and 72 in test 2. For mean comparisons between misted vs nonmisted individual cultivars, LSD 0.05 = 10.9 and 13.9 for test 1 and 2, respectively.

[b]S - susceptible, I - intermediate, R - resistant (based on nonmisted conditions).

Table 3. Disease index, percent infected roots, percent rot per infected root, and yield of sugar beet as affected by Erwinia root rot under sprinkler and furrow irrigation.

		Type of irrigation	
Test (yr)		Furrow	Sprinkler
Test 1, 1973	Disease index[a]	9.1	10.6
	% infected roots	18.8	21.9
	% rot/infected root	49.7	48.0
	Yield, t/ha[b]	81.1	86.5
Test 2, 1973	Disease index	10.9	12.5
	% infected roots	21.2	24.5
	% rot/infected root	52.8	49.9
	Yield, t/ha[b]	78.2	85.5[c]
1974	Disease index	4.8	5.1
	% infected roots	12.1	14.5
	% rot/infected root	39.4	34.5
	Yield, t/ha[b]	40.0	40.0
x	Disease index	8.3	9.4[c]
	% infected roots	17.4	20.3[c]
	% rot/infected root	47.3	44.1
	Yield, t/ha[b]	66.4	70.7

[a]Disease index equals the sum of % rot per beet divided by number of beets per plot.

[b]Metric tonnes/hectare.

[c]Significantly different at P = 0.05, as determined by LSD.

The increase in the DI under sprinkler irrigation was 13.3% compared to furrow irrigation. Increases in the percentage infected roots of sprinkler irrigated beets occurred in the tests conducted in 1973 and 1974 when compared with furrow irrigation. When analyzed over years, this increase (16.7%) was significant at P = 0.05. In both years, the percentage of infected plants of one cultivar was increased significantly (Table 4).

Table 4. Percentage infected roots of sugar beet as affected by cultivar and type of irrigation when inoculated with *Erwinia* sp.

	Percent infected roots under indicated irrigation[a]					
	1973		1974		x	
Cultivar	Furrow	Sprinkler	Furrow	Sprinkler	Furrow	Sprinkler
C17	40.0	42.3	24.5	28.4	21.5	23.6
US H10	25.6	37.0*	10.7	8.0	12.1	15.0
546H3	17.2	21.7	7.1	14.1*	8.1	11.9*
US H7	14.9	17.7	6.1	7.6	7.0	8.4
Maris Vanguard	13.0	18.9				
US 75	14.9	12.5				
Y03	14.4	12.4				
x̄	20.0	23.2*	12.1	14.5	12.2	14.7*

[a]Means of two tests in 1973, one in 1974, and the three tests combined (x); * = significantly different from furrow irrigation at P = 0.05, as determined by LSD.

Type of irrigation had no effect on the amount of rot per infected beet, however, there were significant differences among cultivars in each test (Table 5). The range in percentage rot per infected beet was from 63.5 to 25.1

Table 5. Percentage rot per infected beet as affected by cultivar when inoculated with *Erwinia*.

	% rot/infected beet		
	1973		1974
Cultivar	Test 1	Test 2	
C17	63.5x[a]	69.8x	35.5x
US H10	43.8y	53.5y	20.1y
US H7A	44.1y	40.2yz	13.8y
546H3	25.1z	27.4z	17.1y
Maris Vanguard	48.7xy	50.5y	
US 75	41.6yz	43.3yz	
Y03	47.4xy	56.4xy	

[a]Means within columns followed by the same letter are not significantly different according to Duncan's multiple range test at P = 0.05.

in Test 1 and 69.8 to 27.4 in Test 2 in 1973, and from 35.5 to 13.8 in 1974. Although the amount of rot per beet was less in 1974, the cultivars reacted similarly over the 2 years when compared with the most susceptible cultivar, C17.

Only in one test in 1973 were any of the second order interactions significant. In this case, there was an irrigation x inoculation x cultivar interaction as measured by gross sugar. However, there was no consistent pattern of increases or decreases to suggest an influence of cultivar or type of irrigation.

The spread of the pathogen to the noninoculated plots was significant in both years, 18.7% in 1973 and 3.5% in 1974. However, the amount of infection in the inoculated plots was about the same, 24.8% and 23.1% for 1973 and 1974 respectively.

Sugarbeets inoculated 6 weeks after seeding were significantly more susceptible than 8 and 10 week-old plants inoculated at the same time, as measured by percentage of infection, DI, percentage rot per infected beet, and gross sugar yield.

Highly significant age, cultivar, and age x cultivar effects were shown by the analysis of variance. The decrease in percentage infection with age of plant was greater for the resistant cultivar, C64, when compared with the susceptible cultivar, C17. This interaction is shown by the difference in the slope of the effects due to cultivar (Figure 1).

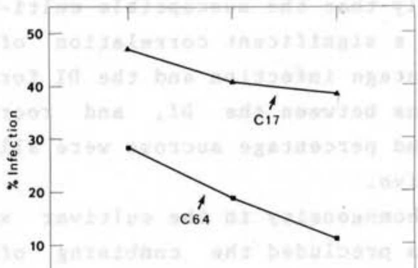

Figure 1. The interaction of age x cultivar as expressed by percent infection of two sugar beet cultivars, C64 (resistant) and C17 (susceptible), when inoculated with an *Erwinia* species at 6, 8 and 10 weeks of age.

The effect of plant age at the time of inoculation on DI was similar to percentage infection, however, an age x cultivar interaction did not occur. Plants were more susceptible to rot at Salinas than at Spence. However, the effects due to age were similar at both locations, but the error variances were not homogeneous; therefore, they are shown separately (Figure 2).

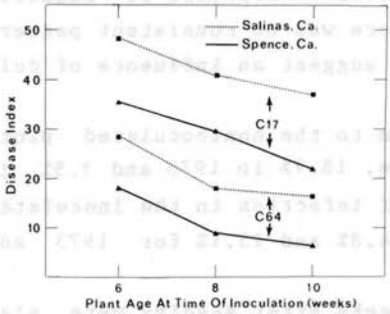

Figure 2. The effect of cultivar and age at two locations (Salinas and Spence, California) on the disease index of two sugar beet cultivars, C64 and C17, when inoculated with an *Erwinia* species at 6, 8 and 10 weeks of age.

Percentage rot per infected beet was similar to the effects measured by the DI. There was a significant effect due to cultivar and age of plants with the resistant cultivar, C64, developing less rot per beet than the susceptible cultivar, C17, (53.8 vs 67.7%) and younger beets were more susceptible to rot than older beets, (6 week, 68.3%; 8 week, 61.8%; 10 week, 52.3%).

Gross sugar yield followed closely the effects of percentage infection. There were significant differences due to age and cultivar and there was a significant age x cultivar interaction. The resistant cultivar increased in gross sugar yield more rapidly than the susceptible cultivar (Figure 3). There was a significant correlation of 0.84 and 0.90 between percentage infection and the DI for the two tests. Correlations between the DI, and root yield, gross sugar yield and percentage sucrose were all highly significant and negative.

Lack of error variance homogeneity in the cultivar x inoculation x injury studies precluded the combining of yield data for years 1976 and 1977 and for locations in 1977. The DI was analyzed over locations but not years.

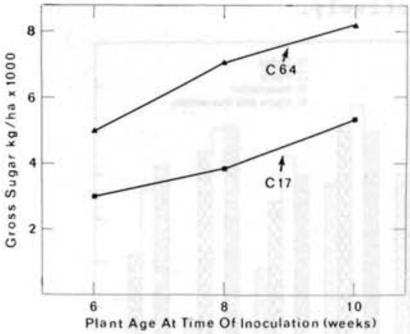

Figure 3. The interaction of age x cultivar as expressed by gross sugar (kg/ha x 1000) of two sugar beet cultivars, C64 (resistant) and C17 (susceptible), when inoculated with an *Erwinia* species at 6, 8 and 10 weeks of age.

At all four test locations a cultivar x inoculation x injury interaction as measured by disease index and yield were shown. Figure 4 and 5 from 1976 are representative

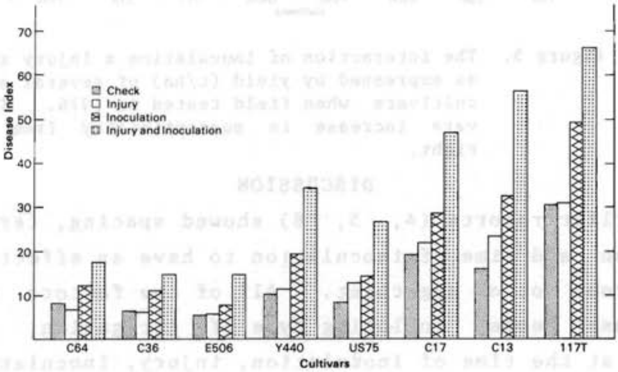

Figure 4. The interactions of inoculation x injury x cultivar as expressed by the disease index of several sugar beet cultivars when field tested in 1976. The cultivars increase in susceptibility from left to right.

of the effects on DI and yield. In general, the more susceptible the cultivar the greater the treatment effect of injury and inoculation. Neither inoculation or injury or the two combined had a significant effect on resistant cultivars; however, on susceptible ones the effect of injury plus inoculation was more than additive (synergistic) for both DI and yield (Figure 4 and 5). Percentage rot per infected inoculated beet varied from 11.0 to 19.4, 28.5 to 37.9 and 51.5 to 74.9 for resistant, intermediate and susceptible beets, respectively.

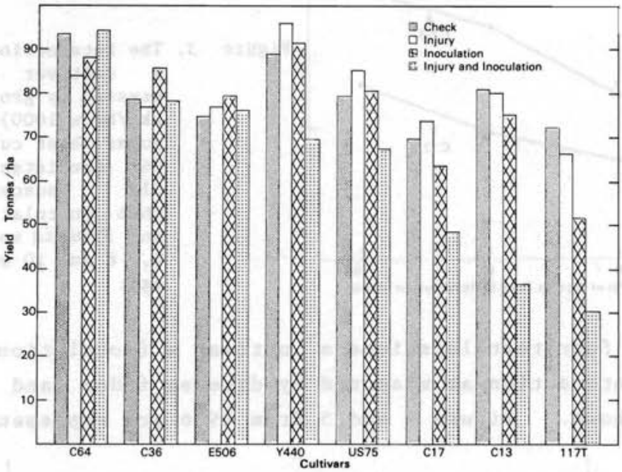

Figure 5. The interaction of inoculation x injury x cultivar as expressed by yield (t/ha) of several sugar beet cultivars when field tested in 1976. The cultivars increase in susceptibility from left to right.

DISCUSSION

Earlier reports (4, 5, 8) showed spacing, fertilizer nitrogen and time of inoculation to have an effect on Erwinia root rot of sugarbeet. All of the factors studied in these tests, including type of irrigation, age of plants at the time of inoculation, injury, inoculation and genotype, and interactions between these factors were found to have an effect on Erwinia root rot of sugarbeet. The interactions resulted from greater effects when two of the factors complimented each other and increased rot more than the additive effects of the two alone (Figure 1, 2, 3, 4 and 5). Of the factors studied in these tests, inoculation had the greatest effect on increasing rot, particularly when injuries were present (Figure 4 and 5). These increased losses suggest that any field operation that would simultaneously injure plants and spread the bacterium, such as cultivation, would increase disease severity.

Because *Erwinia* requires warm temperatures to multiply

(7), early planting when cool soil temperatures prevail would provide older, less susceptible plants when the bacterium becomes most active and thus should be effective in reducing rot. Earlier studies on age of plants (8) substantiate these data, however, those tests were planted at one time and inoculated at different times. Our present tests incorporated different planting dates and one date of inoculation.

Sprinkler irrigation in the field or misting in the greenhouse, increased the susceptibility of sugarbeet to *Erwinia* The data shows that intermediately susceptible cultivar are effected more than highly susceptible or resistant cultivars. Why susceptible cultivars are not effected appreciably is not evident from these tests, however, it may suggest that other factors are more important than length of time injuries are susceptible. For example, moisture may be more important in intermediately susceptible genotypes, because the length of time injuries are susceptible is extended, or that spread by splashing water is more important than in other cultivars. Neither moisture nor injury had any appreciable effect on resistant cultivars, demonstrating that resistance does not break down due to massive injury or the length of time an infection court is susceptible. Splashing of water from sprinkler irrigation appears to effect the spread of *Erwinia* and the amount of rot in the field, but not the amount of rot per infected beet. This is in contrast to other observations that *Erwinia* root rot was greater in furrow-irrigated sugarbeets than sprinkler irrigated sugarbeets in Washington state (3). The fact that infected inoculated beets rot at different rates supports our earlier evidence that a quantitatively inherited system controls rate of root rot (1).

These factors discussed, plus those reported earlier (5, 8), suggest precautions that sugarbeet growers can use to decrease losses from *Erwinia* root rot when adapted resistant cultivars are not available. The most effective control, however, is the use of resistant cultivars if available (1, 9, 10).

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