Bacterial Vascular Necrosis and Rot of Sugarbeet: Genetic Vulnerability and Selecting for Resistance

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We thank M. N. Schroth and S. V. Thomson for supplying the *Erwinia* isolates. We also acknowledge the statistical service of Bruce Mackey.

Accepted for publication 13 October 1977.

ABSTRACT

WHITNEY, E. D., and R. T. LEWELLEN. 1978. Bacterial vascular necrosis and rot of sugarbeet: genetic vulnerability and selecting for resistance. Phytopathology 68: 657-661.

Field and greenhouse selections of sugarbeet for resistance to an *Erwinia* species that incites vascular necrosis and rot have shown high degrees of resistance following two cycles of selection. In all selections tested, resistance for the desired trait was increased. Resistance of hybrids with a selected pollinator as one parent also showed an increase in resistance when compared with hybrids from the same seed parent and the susceptible unselected pollinator. The greater vulnerability to *Erwinia* of the pollen parent of currently grown hybrid sugarbeet cultivars in California appeared to be the result of genetic drift. This drift probably was due to the use of too few plants to reconstitute the gene pool after each cycle of selection for virus yellows resistance.

Additional key words: linkage, cultivars, *Erwinia*.

In 1972, an *Erwinia* sp. was found to be the incitant of a root rot of recently introduced hybrid cultivars of sugarbeet grown extensively in California (4). Since that time, the disease has been reported from most beet-growing areas of California as well as Washington (1, 3), Idaho (D. L. Mumford, *personal communication*), and Arizona (2). Early studies showed that the first epiphytotic recognized in the Lost Hills area of California was partially due to the increased susceptibility of the pollen parents of newly introduced hybrid cultivars (6, 7). Early observations also suggested that sufficient genetic variability existed in susceptible cultivars to allow selection for resistance to the *Erwinia* sp. Further, it was of interest to investigate the possible reasons for the increased susceptibility of these pollen parents since the original parent, US 75, was moderately resistant (5, 6, 7). In this report we describe techniques that were successful in selecting for Erwinia root rot resistance.

MATERIALS AND METHODS

In 1972, 96 sugarbeet roots of C13, the susceptible pollen parent of the hybrid cultivar US H9, were selected from a strip planting in which the incidence of the root rot was about 36% (6). Each beet was split longitudinally and one-half of each beet was placed in a photothermal induction room (6 C, continuous light) for 120 days to induce bolting. The corresponding half of each root was planted in a 20-cm pot of soil and allowed to regrow in the greenhouse. Two and one-half months after replanting, 10 petioles of each beet were injured with a dissecting needle and inoculated (6). The inoculum was obtained as described previously (6). One mo following inoculation the beets were sliced and evaluated for rot. The corresponding photothermally induced beet halves of 13 roots that had remained healthy were planted in a greenhouse isolator chamber to interpollinate and produce polycross seed. Sixteen beets of which the other half had rotted were planted in a separate isolator chamber. Also, 15 roots from field-selected plants only were placed in a third isolator chamber to produce seed (Fig. 1). Four months after planting, the half-sib seed was harvested from each plant.

1973 test.—Six half-sib families from each polycross (Table 1) that produced sufficient seed were selected and planted in 1973 in a randomized complete block design with four replications. Half-sib lines were numbered from E301 to E333. The parent line C13 was planted twice in each block as a check cultivar. The plants were field-inoculated without injury when the plants were 8 and 12 wk old, as described previously (6). Four and one-half mo after planting, the beets were harvested, split, scored for rot, and a disease index (DI) was calculated: DI = (Σ% rot/beet)/(no. beets/plot) (6). A six-increment scale was used: 0, 1, 15, 50, 85, and 100% rot. One hundred forty resistant plants (0 and 1%) from within half-sib lines were selected, cut in half, and corresponding half roots were either transplanted into pots in the greenhouse or induced to bolt.

1974-1975 tests.—The resistant roots of the 140 field-selected roots from the 1973 greenhouse test were planted to produce seed. Seed from roots selected from E302 and E306 were designated E402 and E406, respectively, and were seed composites from 20 plants each showing vascular necrosis (index 1%) but no rot. Roots with vascular necrosis were chosen because this was interpreted as a highly resistant reaction, whereas roots that showed no infection could not be differentiated from possible escapes. The seed composite designated E434

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was produced from plants selected from six half-sib lines (Fig. 1). The plants used to produce E434 also showed vascular necrosis. All plants showing no rot or vascular necrosis were polycrossed and the half-sib seed was harvested individually from each female plant (Fig. 1). Those progenies tested on an individual plant basis are represented by E followed by a hyphenated number; e.g., E402-3 (Table 2).

Included in the isolators of selections from E302, E306, and E334 was a male-sterile F_1 hybrid (546H3) used to produce *Erwinia*-selected hybrid cultivars comparable to US H9 for hybrid evaluations. These 1973 selections, their parents, and hybrids (Table 2) were tested for reaction to *Erwinia* in 1974 in a randomized complete block design with four replications. Procedures used were similar to those used in the 1973 test; i.e., inoculated but not injured. These hybrids, commercial hybrids, and components of hybrids were evaluated for yield and resistance to rot (Table 3); tests were conducted as randomized complete blocks at two locations, Salinas and Spence, California, in 1975. Each test had eight replications. Plants were injured and inoculated to insure infection of susceptible plants (6) 10 wk after seeding. The plants were injured by crushing the leaves and petioles with a device similar to a ski pole constructed from a round 15 cm diameter metal plate attached to a handle. The tests were analyzed statistically to study the influence of selection on yield, DI, and percent infection.

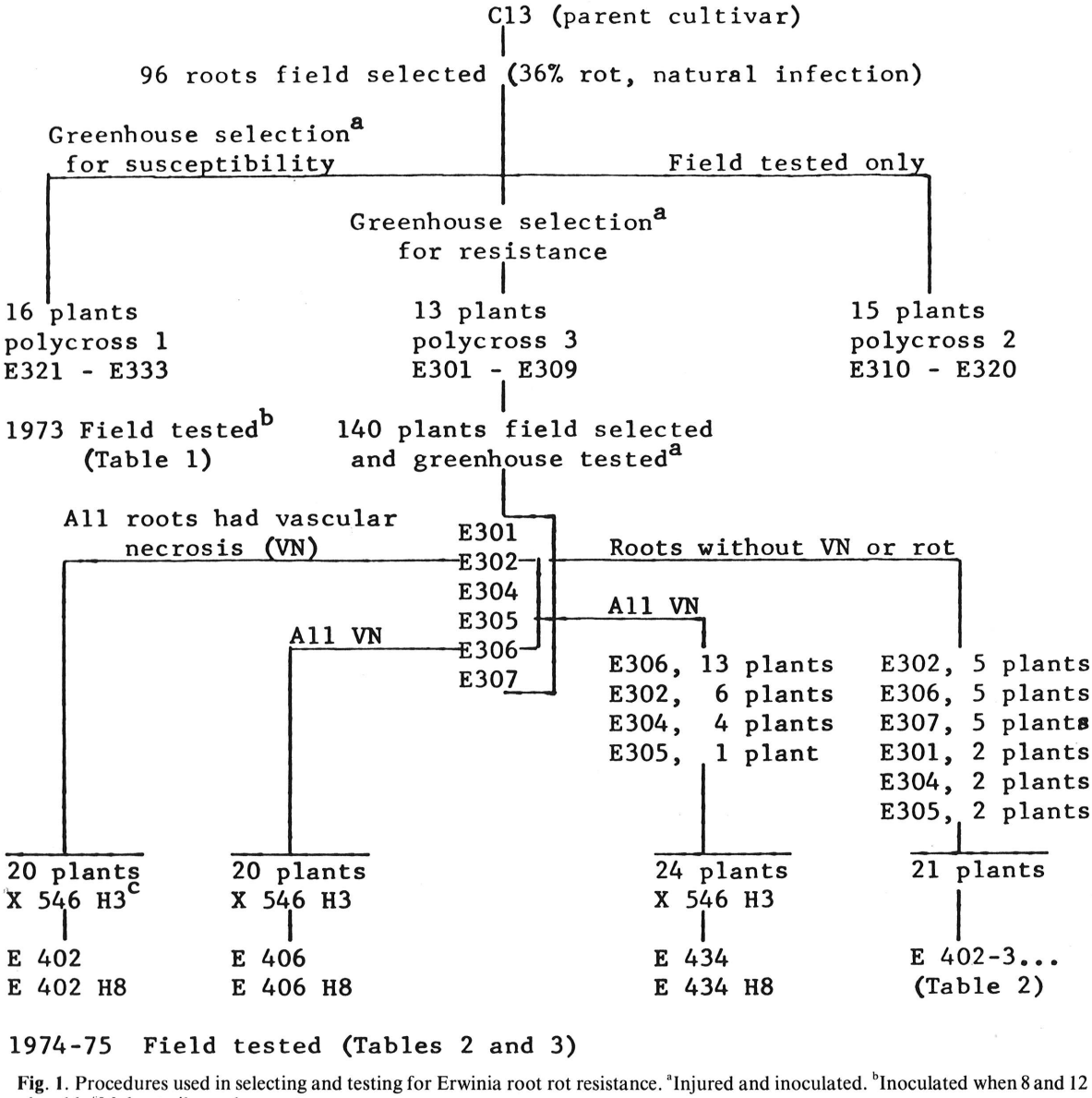

Fig. 1. Procedures used in selecting and testing for Erwinia root rot resistance. [a]Injured and inoculated. [b]Inoculated when 8 and 12 weeks old. [c]Male-sterile seed parent.

TABLE 1. The effect of selecting for resistance to bacterial rot within sugarbeet line C13 on the basis of disease index (DI), Salinas, California 1973

Polycross 1[t]		Polycross 2[u]		Polycross 3[v]		Parental Source	
Selection	DI	Selection	DI	Selection	DI	Parent[w]	DI
E333	51.2[x]a[y]	E318	34.7 def	E301	20.1 ij	C13	43.4
E322	48.3 ab	E310	31.1 efg	E307	18.3 ijk		
E329	47.2 ab	E315	30.7 fg	E305	16.3 jkl		
E324	42.0 bc	E316	27.7 gh	E304	14.3 jklm		
E328	41.9 bc	E314	23.0 hi	E302	9.9 lmn		
E321	37.1 cde	E320	15.2 jklm	E306	4.5 n		
$\bar{x}$[z]	44.6		27.1		13.9		43.4

[t]Selected for susceptibility after one cycle of field selection for resistance.
[u]One cycle of field selection for resistance from plants that were not uniformly inoculated.
[v]One cycle of field selection and subsequent greenhouse reselection for resistance.
[w]Parent of selected material.
[x]Mean of four replications.
[y]Duncan's multiple range test, $P = 0.05$, within and between polycrosses.
[z]Arithmetic mean ($\bar{x}$) for which LSD ($P = 0.05$) = 6.4.

TABLE 2. Mean disease index (DI) of lines selected from sugarbeet line C13 for *Erwinia* resistance, Salinas, California, 1974

Entry	Description	DI
C13	Pollen parent of US H9	20.4 a[y]
546H3	562CMS × 546	6.3 bcde
US H9B	546H3 × C13	9.6 bc
C64	Pollen parent of US H7	7.3 bcd
US H7	546H3 × C64	4.1 cde
E434H8	546H3 × E301,2,4,5,6, & 7	2.7 de
E434[z]	The following selections are from C13. See Fig. 1.	1.7 de
E302[z]		1.7 de
E402		1.3 de
E402-3		1.0 e
E402-4		0.2 e
E402-5		1.3 de
E304		11.1 b
E404-2		0.6 e
E404-3		3.1 de
E404-7		0.9 e
E305		3.9 cde
E405-1		0.3 e
E306		2.0 de
E406		0.8 e
E406-2		1.5 de
E406-3		0.9 e
E406-5		1.0 e
E406-8		0.6 e
E406-9		1.1 e
E406-10		0.3 e

[y]Means with the same letter are not significantly different (P= 0.05) according to Duncan's multiple range test.
[z]*Erwinia*-resistant selection; E3- one cycle of selection, E4- two cycles of selection from C13.

Available ancestors of C13 (Table 4), the susceptible pollinator line of US H9, were tested to determine in which cycle of selection increased susceptibility had occurred. Plants grown in the greenhouse were injured (four petioles) and inoculated when 6 wk old. Plants were evaluated 3 mo after inoculation on a scale of 0 to 2: 0 = healthy, 1 = infected, 2 = dead. Two other easily distinguishable traits, hypocotyl color and yellow leaf fleck, were recorded and tested for independence from disease reaction to determine if there were associations between these traits and susceptibility since it was obvious that they had changed during selection. The test was conducted twice with at least 30 plants per test for each selection.

RESULTS

1973 test.—As calculated from the DI, a linear relationship existed among the 18 half-sib families based on the degree of selection pressure among the half-sib families from the three polycrosses (Table 1). The greenhouse selections for susceptibility, originally selected for resistance under natural field infection, were similar to the susceptible parent. Offspring from all plants selected in the field and subsequently reselected for resistance in the greenhouse were more resistant than the parent C13. All but one of the field selections (E318) were more resistant than the parent (Table 1).

1974 test.—Some improvement in resistance was made in the second cycle of selection but the increment was much less than that obtained after the first cycle of selection (Table 2).

1975 test.—A significant location × cultivar interaction occurred as determined by the DI. Selections E402 and 546H3 reacted somewhat differently at the two locations (Table 3). At both locations, all of the selections and hybrids from selections were significantly more resistant than the parent or hybrids from the C13 pollen parent (Table 3). All the selections and their hybrids had less rot per infected root than that of the highly susceptible parent (Table 3). The correlations between percent rot per

infected beet and percent infected plants for Salinas and Spence were highly significant, 0.89 and 0.80, respectively. With increased resistance, yields were, with one exception (E402), significantly increased for both selections and hybrids from selected parents (Table 3).

Pedigree evaluation of C13.—Of those ancestors with enough remnant seed to test their susceptibility, 313 was the first parent to show an increase in susceptibility (Table 4). This population was an increase from five plants from a polycross population of 142 plants. Parents of 313 were similar in resistance to their parents, but subsequent selections were more susceptible. This was true for both percent of plants with infection and susceptibility as calculated by DI (Table 4). Close associations were observed between hypocotyl color, yellow leaf fleck, and susceptibility (three easily discernable characters) the frequency of which had changed during selection (Table 4).

DISCUSSION

The data show that rapid progress can be made by selecting for bacterial rot resistance in sugarbeet. Our data support other observations (Whitney and Lewellen, *unpublished*) that a single locus with dominance conditions resistance to infection, since progress was rapid and the most progress was made in the first selection cycle.

In the first test (1973, Table 1), all plants selected for susceptibility should have produced susceptible offspring if susceptibility is simply inherited and recessive. This was not the case, suggesting that some resistant plants were judged as susceptible. This could easily happen when resistant split beets died from other causes in the greenhouse test.

Greenhouse tests of several cultivars have not shown a variety × isolate interaction or that races of the pathogen

TABLE 3. Effect of two cycles of selection for *Erwinia* resistance on sugar yield, % infection, disease index (DI) and % rot/infected beet of sugarbeet selections and hybrids tested in 1975 at Spence and Salinas, California

Cultivar	Spence				Salinas			
	Sugar yield[t] (kg/ha)	Inf. (%)	DI	Rot per infected beet (%)	Yield	Inf. (%)	DI	Rot per infected beet (%)
C13[u]	460 d[v]	95.7 d	83.5 d	86.8	1,344 d	86.7 f	74.5 e	84.9
E434[w]	2,231 b	75.1 b	31.6 a	40.7	6,090 ab	35.3 a	14.1 ab	30.9
E406[w]	2,147 c	81.5 bc	31.3 a	38.0	6,050 ab	44.1 abc	12.0 a	27.7
E402[w]	822 d	88.9 cd	62.4 c	67.9	4,416 c	50.2 bc	18.8 ab	35.4
546H3[x]	2,655 ab	60.1 a	26.0 a	43.1	5,055 bc	46.6 abc	22.6 bc	49.6
US H9[y]	1,830 c	82.7 bc	53.6 b	64.4	4,367 c	70.0 e	44.3 d	63.9
E434H8[z]	2,812 a	71.9 b	26.1 a	35.6	6,300 a	40.0 ab	15.4 ab	39.5
E406H8[z]	2,763 a	72.6 b	32.2 a	43.7	6,547 a	37.4 ab	15.5 ab	42.0
E402H8[z]	2,882 a	75.7 b	32.7 a	42.7	5,844 ab	57.1 cd	22.9 bc	40.7

[t]Gross sugar (kg/ha).
[u]Pollen parent of US H9.
[v]Duncan's multiple range test, $P = 0.05$, within each treatment and location.
[w]Selections from C13.
[x]Seed parent of hybrids.
[y]Susceptible hybrid cultivar.
[z]Resistant hybrid cultivar.

TABLE 4. The evaluation of the ancestors of sugarbeet selections developed for yellows resistance to determine when *Erwinia* susceptibility was increased

	Cultivars									
	US 22	US 75	511[a]	711	911	0116[a]	113[a]	313	413C	C13
Disease index	38.0[b]	41.5		43.0	34.0			77.0	65.0	75.0
% Infection	50.0[b]	53.0		36.8	50.0			87.3	79.4	87.0
% Red hypocotyl	81.8[c]	51.5		27.3	24.2			0.0	0.0	0.0
% Yellow leaf fleck	6.0[c,d]	18.2		18.2	30.3			45.5	36.4	60.6
No. plants/seed increase	com[e]	com	62	125	142	5	11	32	com	com

[a]No seed available for testing.
[b]Two tests of at least 30 plants per test.
[c]One test of at least 30 plants per test.
[d]Test of independence was significant, $\chi^2 = 19.6$, $P = 0.05$, which suggests an association between yellow leaf fleck and susceptibility. susceptibility.
[e]Commercial seed increase.

occur (Whitney and Lewellen, *unpublished*). The use of several isolates in the inoculum minimized that possibility since large numbers of isolates have not been tested extensively.

Our conclusion that the use of too few plants to reconstitute the gene pool when making selections for virus yellows resistance may have resulted in genetic drift emphasizes the risk that is involved in making inbreds or using too few plants, in this case five, to reconstitute a population.

Although close associations were suggested between bacterial rot susceptibility, hypocotyl color, and leaf fleck, it was not possible to confirm these associations for hypocotyl color and susceptibility due to too few segregating populations. However, it does appear that leaf flecking is linked to susceptibility (Table 4).

Our data suggest that several overlapping genetic mechanisms may influence the percentage of infected plants and the rate of rotting within infected beets. This is supported by our observations that not only is the percent infected plants decreased by selection, but also the DI and percent rot per infected beet are decreased to a greater extent than rate of infection. This suggests that rate of rotting is reduced by additional factors, which have been concentrated in selections and probably are present also in other cultivars.

The difference in resistance (DI) for the two tests in 1975 suggests that plants may escape infection or that injury provides infection courts for other organisms that incite rot. This emphasizes one of the difficulties in studying the genetics of root-rot resistance in plants.

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