A Comparison of Several Methods of Testing Sugar Beet Strains and Individual Roots for Resistance to Storage Pathogens[1]

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Certain inoculation techniques for testing storage-rot resistance of sugar beet roots *(Beta vulgaris* L.) have been described by workers in the United States in recent years (2, 4, 5)[3], and progress in breeding for improved storage rot resistance also has been reported (3). However, the need for further information regarding inoculation techniques has been apparent in at least two respects. First, it was clear that a reliable method was needed for testing small plant populations and, second, more efficient means of screening individual mother-beet roots for resistance were much to be desired.

Methods

In 1952 eight sugar beet strains (Table 1) representing a wide range in storage-rot resistance, as determined in earlier experiments, were grown in Latin-Square arrangement in a field near Eaton, Colorado. The crop was cared for in the usual way. At harvest, October 6-9, two representative samples of approximately 20 beets each were taken from each of the 64 field plots, topped, injured further by a uniform procedure, washed, and stored without artificial inoculation—one sample at 65° F. and the other at 45° (± 2° F.). Storage conditions were controlled as described in an earlier report (1). The duration of storage was approximately 12 and 21 weeks for the 65° and 45° temperatures, respectively. At the end of storage the percentage of rotted tissue was determined for each sample by separating and weighing the healthy and rotted portions. The percentage-rot data were summarized and analyzed with logarithmic transformation.

Representative roots for inoculation purposes also were taken from each field plot at harvest. They were trimmed as mother beets, washed, and held in crates until needed for inoculation. Storage temperatures for these roots prior to inoculation averaged about 42-45° F., relative humidity was high, and the roots remained in good condition.

The following fungus cultures were used in the inoculation trials: *Botrytis cinerea* Fr., A and A'; *Phoma betae* Frank, B and B'; and *Penicillium* sp., C. All five cultures had been isolated from sugar beet storage-rot specimens. The first two species named were emphasized in the selection of cultures since they were considered the most important pathogens in commercial sugar beet storage piles in northern Colorado.

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[3] Numbers in parentheses refer to literature cited.

Table 1.—Description of Sugar Beet Varieties or Str;

Temporary Strain No.	Fort Collins Seed No.	Description
1	471004-0	U. S. 226 (synthetic commercial variety; leaf-spot resistant).
2	511803-00	Storage-rot-resist ant selection from U. S. 226.
3	Acc. 1181	G. YV. 304-50A (commercial variety; leaf-spot resistant.)
4	Acc. 5072	U. S. 22/3 (commercial variety; curly-top resistant).
5	Acc. 1139	S. L. 1-300 (Increase of R and G Old Type).
6	471003-0	High-yielding inbred.
7	461020-0	High-yielding inbred.
8	Acc. 1216	High-yielding inbred.

Inoculation techniques studied were as follows:

1. Agar inoculum placed on freshly exposed surface of transverse slice of tap root as described in an earlier report (4) ; slice approximately 1 inch thick.

2. Agar inoculum applied to a small area exposed by removal of the epidermis from section of tap root.

3. Spore-suspension inoculum, prepared with 1:1000 Dreft solution, placed on transverse slice as in method 1.

4. Sterile, wooden toothpick dipped in spore suspension (1:1000 Dreft solution) and driven approximately 1 inch into the tap root.

Any one inoculation trial—i.e., one fungus culture and one technique at a given date—involved two sugar beet roots from each of the 64 field plots, with duplicate inoculations for each root. Two sets of inoculation trials were conducted—one begun December 15-16, 1952 and the other March 26-27, 1953. Methods 1 and 2 were used in the former, and 1, 3, and 4 in the latter. Following inoculation, specimens were held at 45° F. (zt 1°)

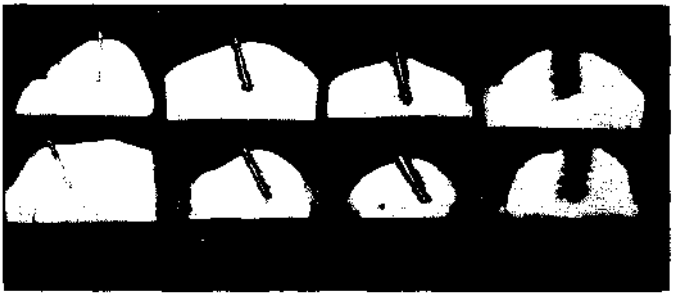

Figure 1. Cross-sectional view of sugar beet roots showing varying reaction to *B. cinerea* toothpick inoculation. The pair of slices at the left represents **two control roots. The other three pairs, from left to right, represent three respective roots which had been inoculated in duplicate and ranged from resistant to very susceptible. All roots were held for 25 days at 45° F. after the toothpicks were inserted.**

for 25 to 42 days with relative humidity between 97 and 100 percent, approximately. Depth of rotted tissue was measured for each inoculated spot for methods 1, 2, and 3. For method 4 the average width of the rotted section surrounding the toothpick was recorded, with the root cut transversely as illustrated in Figure **1.**

Results

The percentage rot data obtained from the samples which were not inoculated artificially failed to show significant interaction of beet strains x temperatures, and the results were combined into the single set of averages shown at the left in Tables 2 and 3. As indicated by the F-test, highly significant differences occurred between strains. Laboratory platings made from rotting specimens taken from comparable samples near the end of storage indicated that *B. cinerea* and *P. betae* were the principal pathogens affecting the uninoculated roots. No attempt was made to appraise the relative importance of the two. The latter occurred with greater frequency in the plates, but a tendency toward more severe rotting action was noticed for the former, and both were considered important.

Table 2-—Storage Rot in Uninoculated Samples of Eight Sugar Beet Strains and in Comparable Root Tissue Specimens Inoculated by Two Methods, December 15-16, 1952.

		Fungus Cultures, Time, and Average Depth of Rotted Tissue[2]					
		Inoculation Method 1			Inoculation Method 2		
Sugar Beet Strains	Rot in[1] Uninoculated Samples (Geom. Mean)	A (Botrytis) 32 Days	It (Phoma) 32 Days	C (Penicillium) 34 Days	A (Botrytis) 42 Days	B (Phoma) 42 Days	C (Penicillium) 42 Days
	Percent	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.
1	2.89	7.5	4.8	2.6	0.7	0.7	0.05
2	1.73	6.1	5.7	2.6	0.3	2.4	0.25
3	2.07	6.6	3.9	2.5	0.8	1.2	0.23
4	4.42	7.0	4.4	3.8	1.7	1.8	0.16
5	3.08	6.6	5.0	3.6	2.3	1.7	0.06
6	7.03	10.3	3.8	2.5	2.7	0.7	0.08
7	7.03	10.2	4.5	2.9	1.7	0.5	0.08
8	6.46	14.5	8.0	3.4	1.2	2.9	0.25
General imean		8.59	5.01	2.97	1.40	1.52	0.14
F	4.55	6.73"	3.55"	0.68	1.10	1.08	[3]
C.V.		36.7	40.5	61.3	157.4	144.4	**3**
L.S.D. (5 percent point)		3.2	2.0	1.8	2.2	2.2	**3**
r[4]		0.81[5]	0.13	0.16	0.62	— 0.19	—0.25

[1] 65° and 45° F. combined; total of approximately 320 roots per strain.
[2] Each strain average based on 16 roots (32 inoculations); temperature 45° F.
[3] Variance analysis was omitted due to the relatively high frequency of zero determina-

[4] Correlation of the indicated column with rot percentages for uninoculated samples.
[5] — Odds at least 19:1.
[6] — Odds at least 99:1.

Summarized results for the December and March inoculation trials are presented in Tables 2 and 3, respectively. Considering, first, the significance of differences between beet strains (as measured by F) , the coefficient of variability (C.V.) , and the relative degree of pathogenic action, certain observations seem to deserve special mention:

1. Method 2 appeared to be unsatisfactory in each of the three trials in which it was used. *In* the case of *Penicilhum* sp., although variance analysis was not made, erratic results and frequent occurrence of zero determinations were sufficient to reject the method insofar as that particular culture was concerned. The method 2 trials involving *B. cinerea* and *P. betae* failed to show significant differences between strains, and the C. V.-values were extremely high.

2. In the only other trial involving *Penicillum* sp. (method 1, culture G) , the results also were unsatisfactory as evidenced by low F and high C. V.

3. In every trial in which *B. cinerea* or *P. betae* was used, with the exception of method 2, the F value showed that significant differences occurred between strains. However, because of higher G. V. values, method 3 appeared to be less desirable than 1 and 4.

Table 3.—Storage Rot in Uninoculated Samples of Eight Sugar Beet Strains and in Comparable Roots and Root-Tissue Specimens Inoculated by Means of Three Methods, March 26-27, 1953.

Sugar Beet Strains	Rot in[1] Uninoculated Samples (Geom. Mean)	Fungus Cultures, Time, and Average Rot Measurements[2]							
		Inoculation Method 1				Inoc. Method 3		Inoc. Method 4	
		Botrytis		Phoma		Botrytis	Phoma	Botrytis	Phoma
		Cult. A 25 Days	Cult. A' 25 Days	Cult. B 26 Days	Cult. B' 25 Days	Cult. A' 25 Days	Cult. B' 25 Days	Cult. A' 26-27 Days	Cult. B' 26-27 Days
	Percent	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.
1	2.89	10.3	12.0	8.9	6.8	9.0	1.3	10.9	3.5
2	1.73	8.1	8.5	7.5	5.7	4.3	0.8	7.6	3.0
3	2.07	9.2	9.3	6.7	5.5	6.9	0.6	9.3	3.6
4	4.42	9.1	10.5	9.4	7.6	7.4	0.7	7.8	2.7
5	3.08	9.2	10.9	8.7	6.3	6.2	0.7	9.2	4.4
6	7.03	13.5	14.1	8.0	6.7	8.8	0.7	10.5	3.7
7	7.03	10.3	11.7	8.2	6.9	7.8	1.5	10.6	3.1
8	6.46	14.1	14.6	11.7	9.8	16.8	2.5	12.3	4.2
General mean		10.46	11.45	8.62	6.91	8.38	1.09	9.77	3.51
F	4.55"	4.48[4]-	2.98[5]	3.42[h]	3.59"	6.63"	2.72"	2.95"	3.31"
C.V.		27.8	30.9	26.3	28.9	48.8	103.6	27.1	25.4
I..S.D.'		2.9	3.6	2,3	2.0	4.1	1.1	2.7	0.9
r[1]		0.77-"	0.81[s]	0.45	0.61	0.57	0.50	0.61	0.09

[1] 65° and 45° F. combined; total of approximately 320 roots per strain.
[a] Each strain average based on 16 roots (32 inoculations); temperature 45° F. Measurements for method 4 represent average width of rotted section surrounding the toothpick; all other measurements represent depth of rotted tissue.
[3] Five percent point.
[4] Correlation of the indicated column with rot percentages for uninoculated samples.
[3] = Odds at least 19:1.
« — Odds at least 99:1.

The following highlights observed in comparing the sugar beet strain averages obtained from inoculation trials with the strain averages obtained from uninoculated samples are of interest:

1. In general the results from trials with *B. cinerea* agreed much more closely with the uninoculated samples than did the data from *P. betae* inoculation.

2. In each of the three trials in which *B. cinerea* was used with inoculation method 1, the r value was significant (0.81, 0.77, and o.81, respectively) .

3. The strain averages obtained for method 4, with *B. cinerea,* agreed fairly well with those for the uninoculated samples, the r value (0.61) approaching significance.

4. The results for method 4, with *B. cinerea,* agreed quite closely with those obtained for the comparable trial under method 1 (Table 3, culture A') . The r-value in this case, 0.85 (not shown in table) , was highly significant.

The behavior of strains 1 and 2 in the inoculation trials is of special interest. Strain 1 is U. S. 226, a wide-base synthetic variety. Strain 2 was developed from U. S. 226 by two generations of selection for storage-rot resistance. In each of the six inoculation trials involving *B. cinerea,* strain 2 was below the parental variety in average rot measurement. The difference was significant in two trials, approximately so in a third, and general averages based on all six trials showed strain 2 to be 31 percent below No. 1. These data strongly indicate that strain 2 is superior to the parental variety in resistance to *B. cinerea.* The results are in agreement with those from uninoculated samples, though the difference between the two strains in the latter comparison was not significant. In contrast with the findings for *Botrytis,* the difference between strains 1 and 2 in reaction to *P. betae* was negligible. In the 6 trials in which the latter species was used, none of the mean differences between those strains was significant, and the two general strain averages were practically identical. From these data it seems clear that improvement in resistance to *B. cinerea* by selection is feasible, in the U. S. 226 variety, and that such improvement does nor necessarily constitute better resistance to *P. betae.* _ This apparent independence of resistance to the two fungi is in keeping with the contrasting correlations discussed above.

Evidence indicating that resistance decreases during storage may be seen in Tables 2 and 3, cultures A and B, inoculation method 1. The rate of rotting as measured by the general mean was 0.27 mm. per day for *Botrytis*-culture A when inoculations were performed in mid-December, 1952. The corresponding mean for the series of inoculations performed late in March, 1953, was 0.42, an increase of 56 percent. Similar results were obtained for P/zoma-culture B, the average for March inoculations (0.33 mm. per day) being more than double the rate for the December trial.

Summary

An experiment was conducted primarily for the purpose of studying inoculation methods for testing sugar beet strains and individual roots for resistance to storage pathogens. Roots of eight strains grown in replicated field plots, were injured uniformly at harvest and stored without artificial inoculation under controlled conditions—a total of approximately 320 roots per strain. At the end of storage the percentage of rotted tissue was determined for each sample.

Comparable roots from the same field plots were inoculated in four different ways using a total of five fungus cultures representing three species.

Fourteen inoculation trials were conducted, each trial at a given date involving a single fungus culture, one method of inoculation, and a total of 16 roots (32 inoculations) for each sugar beet strain. The amount of rot was determined by measurement several weeks after inoculation.

Highly significant differences between beet strains, in percentage of rotted tissue, occurred in the set of uninoculated samples as shown by an F value for strains in excess of the 1 percent point.

Appraisal of precision by means of the F test indicated that, for *B. cinerea* and *P. betae* inoculations with methods 1 and 4, samples of 16 roots per strain were nearly as effective as were samples of approximately 320 uninoculated roots as a means of showing the occurrence of significant differences between strains.

The average rates of rotting for the 8 respective sugar beet strains in each of the three *Botrytis* inoculation trials in which method 1 (interior agar technique) was used agreed quite well with the percentage-rot averages obtained from uninoculated samples (significant r values 0.81, 0.77 and 0.81, respectively) . The results obtained for *Botrytis* with method 4 (toothpick-spore-suspension procedure) agreed fairly well with the uninoculated samples (r 0.61) and closely paralleled the comparable results for method 1 (r 0.85, highly significant) . The data obtained from *Phoma* inoculations, in general, did not agree closely with the uninoculated samples.

Strain 2 which had been selected for storage-rot resistance from U. S. 226 (strain 1) , was substantially superior to the parental variety in resistance to *B. cinerea*, but there was essentially no difference between the two strains in reaction to *P. betae.* These results indicated (a) that improvement in storage-rot resistance, by selection, is feasible insofar as *B. cinerea* is concerned; and (b) that improved resistance to that pathogen does not necessarily constitute better resistance to *P. betae.*

Comparable inoculations performed in December, 1952, and in the following March, using *B. cinerea* and *P. betae,* showed considerable loss in resistance to each fungus during the intervening period, in spite of the fact that the roots were held under conditions generally considered quite satisfactory for mother beet storage.

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