A Study of Two Methods of Testing Individual Sugar-Beet Roots for Resistance to Storage Pathogens[1]

JOHN O. GASKILL[2]

The feasibility of improving storage-rot resistance of sugar beets *(Beta vulgaris* L.) through breeding has been indicated by results presented in other reports (1, 2, 7)[3]

A method described by the author for selecting resistant mother beets (1) involved wound inoculation of the tap roots with composite inoculum, storage for five weeks at 45° F., and subsequent examination for depth of rot by means of a transverse cut through the inoculated area on each root. Those mother beets in which depth of rotted tissue was the least were cut off at the point of examination and transplanted in the field for seed production. This procedure was used on the following tentative assumptions: 1. that many of the more susceptible individuals could be eliminated by examination at the end of the five weeks' storage period; 2. that further elimination of susceptible plants would occur automatically in the field after transplanting, and *3.* that, of the surviving individuals, those producing the largest quantities of seed would tend to be the most resistant.

No attempt has been made to confirm these assumptions individually. However, the net result of application of the method in the U. S. 226 variety was substantial improvement in storage-rot resistance (2) .

When it is desirable to subject each individual mother beet to various chemical analyses in addition to pure-culture inoculation by more than one storage pathogen, an inoculation technique involving relatively small tissue specimens would be more practicable than the procedure outlined above. In March, 1950, an experiment was begun for the purpose of studying this problem. Six tissue specimens were cut from the mid-region of the tap root of each of 55 comparable mother beets of the wide-base, leaf-spot-resistant variety, U. S. 226, leaving a portion (of crown and tap-root tissue) in condition suitable for seed production. The six specimens were washed in cool tap water and distributed among three incubation chambers, one large and one small specimen in each chamber. Semi-fluid potato-dextrose-agar inoculum was preparted for each of three cultures of storage pathogens—two of *Phoma betae* Frank* and one of a species of *Botrytis*[5]. All

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[2] Plant Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Fort Collins, Colorado. Acknowledgment is made to the Beet Sugar Development Foundation for facilities and to the Botany and Plant Pathology Section, Colorado Agricultural Experiment Station, for facilities and for two of the fungus cultures used in these investigations; also to Prof. A. G. Clark, Head of Mathematics Department, Colorado A & M College, for advice regarding statistical methods.

[3] Numbers in parentheses refer to literature cited.

[4] Furnished by Dr. Ellis F. Darley, formerly Assistant Plant Pathologist, Botany and Plant Pathology Section, Colorado Agricultural Experiment Station.

[5] After this article had been completed, the latter culture was identified as *Botrytis cinerea* Fr. by Dr. Carl E. Seliskar, Assistant Plant Pathologist, Botany and Plant Pathology Section, Colorado Agricultural Experiment Station.

tissue specimens in each of the three chambers were inoculated with one of the three respective batches of inoculum, as follows (see Figure 1) :

1. Large pieces—"interior type" inoculation: A thin transverse slice was removed from each specimen, and two drops of inoculum were placed separately on the freshly exposed surface. Inoculated surfaces were comparable with respect to original position in the tap root.

2. Small pieces—"peeled surface" inoculation: Using an ordinary vegetable peeler, the periderm and underlying tissue were removed to a depth of approximately 1 to 2 mm., and two drops of inoculum were applied separately to the peeled surface.

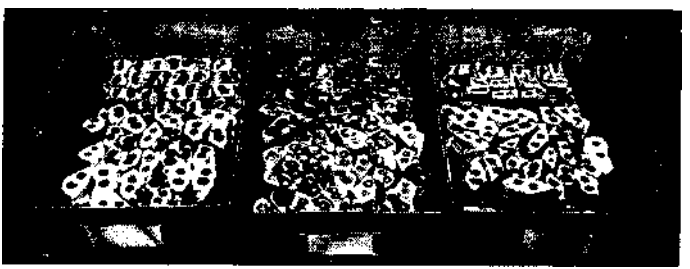

Figure 1. Incubation chambers, with covers removed, showing sugar-beet root-tissue specimens 27 days after inoculation—interior inoculation method in foreground and peeled-surface method in background- A, *Phoma betae* (culture A-2); B, *Botrytis* sp. (culture 5-1); and C, *P. betae* (culture A-17). The size of the specimens may be visualized by comparison with the width of the chambers (13 inches).

Following inoculation, the specimens were returned to the incubation chambers where they were supported on galvanized wire shelves, over water, for 28 days at 45° F., with relative humidity approximately 100 percent. The chambers were covered loosely. At the end of the 28-day period the depth of discolored tissue beneath each inoculated spot was measured.

The procedure described was used in this experiment in preference to the Russian workers' petri-dish-*Botrytis* technique described by Savitsky (7), partly because of convenience and partly because of the desire to employ a temperature more nearly equivalent to the normal temperature inside commercial storage piles. The use of *Phoma betae* was based on reports of Larmer (5) and others indicating the leading role of that pathogen in causing sugar beet storage rots in the United States. *Botrytis* was included because of its prevalence in earlier storage experiments conducted by the author at Fort Collins and because of the importance of that genus—the *cinerea* type in particular—as a sugar-beet storage pathogen elsewhere, especially in England and Russia (3, 4, 6).

Results

The fungi used for making the inoculations were recovered consistently from representative specimens by means of laboratory platings. Negligible contamination was indicated. The appearance of the specimens at the end of the storage period is illustrated in Figures 1 and 2, and the results are summarized in Table 1. Conclusions of special interest which may be drawn from the interior-inoculation results include the following:

1. Individual roots differed very significantly in average rate of rotting.

2. As shown by correlation figures, roots resistant to one culture tended to be resistant to the others, and vice versa. This was in keeping with results obtained by certain Russian workers, as reported by Savitsky (7). However, the occurrence of definite exceptions to this trend is shown by the very significant interaction, cultures x roots.

3. The *Botrytis* culture was much more virulent than either culture of *Phoma,* the differences in depth of rot being highly significant.

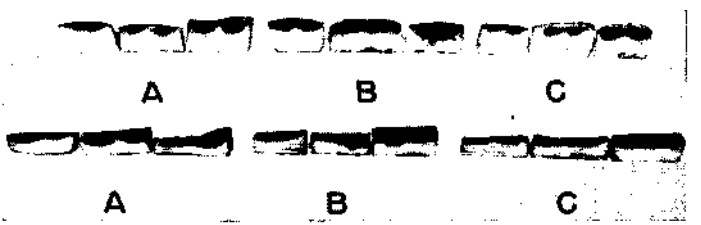

Figure 2. Top—Halves of representative specimens of sugar-beet root tissue showing depth of rot 32 days after **inoculation by the interior method: A, *Phoma betae* (culture A-2), three roots; B, *Botrytis* sp. (culture 5-1), three roots; and C, *P. betae* (culture A-17), three roots. Bottom—rot resulting from peeled-surface type of inoculation; otherwise similar to top view. Note differing reaction of individual roots within each group of three (the roots represented by any one group are not necessarily the same as those of any other group).**

The average rate of rotting resulting from the peeled-surface method of inoculation was about half that obtained for the interior method. The results from the former were more erratic, as indicated by the higher coefficient of variation, but led to similar conclusions. Positive correlations between the two methods were significant, though exceptions to the general trend are apparent in the table.

From the results presented it seems probable that either of the **two** techniques described could be used satisfactorily as a means of testing mother-beet roots for storage-rot resistance. The interior method of inoculation was used, with one culture each of *Botrytis* and *P. betae,* for screening several hundred mother beets in the spring of 1950. Progeny tests, now under way, will be used to evaluate the usefulness of this technique.

Table I.—Depth of Rot in Sugar Beet Tissue Specimens 28 Days After Pure-culture Inoculation; the Specimens Were Held at 45° F. with Relative Humidity Approximately 100 Percent.[1]

Root No.	Interior inoculation Cultures and depth of rot[1]				Peeled-surface inoculation Cultures and depth of rot[1]			
	5-1 (Botrytis)	A-17 (Phoma)	A-2 (Phoma)	Aver.	5-1 (Botrytis)	A-17 (Phoma)	A-2 (Phoma)	Aver.
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
1930	14.5	10.5	12.8	12.6	6.5	5.0	6.3	5.9
1931	11.5	10.3	10.3	10.7				
1933	12.0	11.5	15.3	12.9	5.0	1.8	6.8	4.5
1934	10.0	10.0	7.5	9.2	6.5	7.5	4.8	6.3
1935	10.5	8.5	8.5	9.2	5.5	2.5	3.0	3.7
1940	14.0	9.8	8.8	10.8	8.5	4.8	4.5	5.9
1941	9.0	5.8	8.5	7.8	2.3	2.3	3.8	2.8
1942	10.0	9.0	10.5	9.8	5.5	4.0	4.0	4.5
1943	10.0	7.8	8.3	8.7	5.5	3.8	4.0	4.4
1944	15.0	7.5	8.8	10.4	5.0	2.5	2.5	3.3
1945	11.5	8.0	11.0	10.2	7.0	2.0	1.8	3.6
1948	12.5	10.0	7.8	10.1	5.0	4.8	4.3	4.7
1949	14.5	5.3	8.0	9.3	3.0	1.3	3.3	2.5
1951	9.0	7.3	6.8	7.7	4.5	4.8	8.5	5.9
1952	16.0	9.0	10.8	11.9	7.0	2.8	3.3	4.3
1954	12.5	8.3	5.8	8.8	5.0	4.0	6.8	5.3
1955	15.0	9.8	8.5	11.1				
1956	11.0	9.8	8.8	9.8	5.5	2.8	4.5	4.3
1958	13.5	9.3	10.5	11.1				
1962	14.5	9.0	12.5	12.0	10.0	6.5	5.5	7.3
1963	13.0	8.5	10.3	10.6	4.0	4.3	2.8	3.7
1964	14.5	9.5	11.5	11.8	9.0	4.8	3.5	5.8
1965	15.0	4.3	7.3	8.8	7.0	1.8	3.3	4.0
1966	16.0	7.8	9.5	11.1				
1971	13.5	7.8	8.3	9.8	4.0	3.0	4.5	3.8
1972	12.5	9.3	9.8	10.5				
1973	9.5	9.5	10.5	9.8	6.0	4.0	5.0	5.0
1974	12.5	9.0	10.3	10.6	7.0	2.5	4.5	4.7
1976	19.0	10.8	8.3	12.7	9.0	9.5	4.8	7.8

[1] Table 1 continued on page 579.

Summary

Slices from 55 comparable sugar beet roots of the commercial variety U. S. 226 were inoculated in two ways, using two cultures of *Phoma betae* and one of a species of *Botrytis*, and held for 28 days at 45° F. At the end of that period the depth of rotted tissue was measured. Conclusions drawn from inoculation method 1—interior type—included the following: (a) roots differed very significantly in average rate of rotting; (b) roots resistant to one of the three fungus cultures tended to be resistant to the others, and vice versa, though there were definite exceptions; and (c) the *Botrytis* culture was much more virulent than either culture of *P. betae*. Average depth of rotted tissue resulting from method 2—inoculum applied on a peeled surface near the exterior of the tap root—was about half that obtained from the interior method. The results from method 2 were more erratic but led to similar conclusions. Positive correlations between methods were significant, but exceptions to the general trend were apparent.

From the results presented it seems probable that the techniques described are suitable for storage-rot-resistance selection purposes. Confirmation of this tentative conclusion will depend on progeny tests.

Table 1 (Continued)

Root No.	Interior inoculation Cultures and depth of rot[1]				Peeled-surface inoculation Cultures and depth of rot[1]			
	B-1 (Botrytis)	A-17 (Phoma)	A-2 (Phoma)	Aver.	B-1 (Botrytis)	A-17 (Phoma)	A-2 (Phoma)	Aver.
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
1977	16.0	9.3	9.8	11.7	9.0	4.5	5.3	6.3
1978	12.5	11.0	9.3	10.9	8.5	4.5	4.3	5.8
1979	13.5	9.3	10.0	10.9	8.0	6.3	3.8	6.0
1981	9.5	6.5	5.8	7.3	7.0	4.0	3.8	4.9
1983	10.5	9.3	7.5	9.1	8.0	4.8	2.5	5.1
1984	13.5	6.3	8.5	9.4				
1985	13.5	6.5	9.3	9.8	2.5	4.5	4.3	3.8
1986	22.0	15.0	9.3	14.8	7.0	4.5	4.3	5.3
1990	9.0	6.5	7.5	7.7				
1991	16.5	12.3	10.0	12.9	8.5	4.0	5.0	5.2
1993	12.5	10.3	8.0	10.3	8.0	5.0	3.5	5.5
1994	10.0	6.8	6.5	7.8	6.5	2.8	4.5	4.6
1997	20.0	14.3	13.0	15.8				
1999	10.0	8.0	9.3	9.1	7.0	4.5	4.8	5.4
2001	10.0	5.5	6.0	7.2	6.0	3.0	2.8	3.9
2006	12.0	7.3	10.0	9.8				
2008	18.5	5.0	14.0	12.5				
2009	10.0	7.8	8.8	8.8	8.5	4.0	4.8	5.8
2010	15.0	12.3	10.3	12.5	9.0	13.0	11.5	11.2
2011	15.5	11.3	10.0	12.3	7.0	3.5	3.8	4.8
2012	12.5	6.8	8.0	9.1	4.0	2.3	1.5	2.6
2039	10.5	8.3	8.8	9.2	7.0	3.5	2.8	4.4
2042	10.5	7.0	5.8	7.8	6.5	3.5	2.0	4.0
2046	12.5	9.0	11.0	10.8	7.0	10.3	3.3	6.8
2054	11.5	7.5	10.5	9.8	6.0	4.0	3.0	4.3
2069	15.0	6.0	9.0	10.7	8.0	5.0	3.8	5.6
Average	12.99	8.66	9.28	10.31	6.45	4.31	4.23	4.99
C. V. (percent)			25.0			42.8		
L.S.D.[2] for comparing:								
Culture means			0.69			0.65		
Root means			2.9			2.5		
F-values for:								
Cultures			90.87**			31.37**		
Roots			2.92**			2.94**		
Interaction, cultures x roots			3.06**			5.20**		
Correlations (r) between cultures:								
Phoma vs. *Phoma*			0.415**			0.509**		
Phoma (av.) vs. *Botrytis*			0.480**			0.384**		
Correlations (r) between methods of inoculation:								
Phoma (aver.)				0.387**				
Botrytis				0.302*				

[1] Basic data are given as averages of two measurements, each of which was obtained from a different inoculation. In certain specimens inoculated by the peeled-surface method, the extent of rotted tissue was such that no accurate measurement could be made. In each such case the root concerned was deleted from the set of results for that type of inoculation.

[2] Five-percent point.

Significance of F and r values:

* = Odds at least 19:1.

** = Odds at least 99:1.

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