Preliminary Studies Applicable to Selection for Low Respiration and Resistance to Storage Rots of Sugar Beets

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The loss of sucrose during storage is largely the result of respiration by the beet and/or rot caused by micro-organisms. These losses become particularly apparent in areas where piling and storage of beets is a common practice. This problem is such that it presents a challenge to all which might aid in reducing the losses. Certainly the plant breeder is not outside the sphere of responsibility in this matter should differences in storage quality exist between sugar beet varieties, or between individual beets of these varieties. Methods adaptable to individual root analysis for both respiration and rot and some results of variations in measurements obtained are presented in this paper.

Methods and Results on Respiration

Respiration rates were compared from measurements of carbon dioxide produced by a given amount of sliced tissue over a given period at a given temperature.

Sample preparation—The procedure employs 15 grams of sliced tissue from the tail of the beet. Slicing is done with a rotary vegetable slicer set to give slices 1/12 inch thick. A section from the tail of the beet having a diameter of approximately 1 1/2 inch is used regardless of the over-all size

Table 1.—Respiration Rates at 20° C. on Sliced Tissues of Five Beets of Two Varieties Tested in Duplicate—Values are Calculated to mg. CO_2 Per kg. Tissue Per Hour.

Variety	Beet No.	Sample 1	Sample 2	Variety	Beet No.	Sample 1	Sample 2
GW305	1	159	168	GW381	1	282	295
GW305	2	172	183	GW381	2	251	259
GW305	3	191	177	GW381	3	266	266
GW305	4	196	196	GW381	4	261	253
GW305	5	248	200	GW381	5	248	240
GW305	Mean	189		GW381	Mean	269	

of the beet. Reasonably comparable transverse discs are thus obtained from each beet, though emphasis is based on weight of tissue rather than on number, size or shape of discs. After weighing, a sharpened nail is forced through the discs constituting the sample and the discs are separated so that all cut surfaces are exposed to the air. The tip of the nail holding the sample is then inserted into the underside of a No. 12 one-hole rubber stopper fitted with a capillary tube.

Measurement of CO_2—The No. 12 stopper and sample are inserted in a 200 cc. wide-mouth specimen bottle immediately after the addition of a known amount of alkali. Upon insertion, the stopper seals the bottle and suspends the tissues in an entirely closed chamber except for an oxygen

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inlet provided by the capillary tube. Oxygen is supplied from an oxygen-filled balloon through a manifold made of T-tubes connected in series, one T-tube being required for each bottle. For absorption of the carbon dioxide, 25 cc. of approximately 0.2 normal barium hydroxide has been used per sample. Blanks, including 25 cc. alkali but no sample, were included at intervals. For back-titration 0.2 normal sulfuric acid was used. For 15 grams' tissue and 19 hours' incubation at room temperature these solutions were convenient. Continuous shaking was maintained during incubation by means of a mechanical shaker making 90 1/2-inch oscillations per minute. Phenolphthalein was used as indicator for titration. Taking the difference in titration value between a sample and its appropriate blank, the amount of carbon dioxide produced by respiration was calculated for this report. For selection purposes, the acid titration values were used without further calculation so long as the blank titration values remained constant.

Results—During the winter season, 1950-51, 116 respiration analyses were run per day on individual beets. This number could be increased if necessary. While it is too early to make a complete report, the results obtained to date should be of interest. Good differential results between beets have been obtained so that selection was not difficult. Reported in Table 1 is a sample of the preliminary work which was made to test method.

Table 2.—Respiration Rates at 25° C. on Sliced Tissue from Ten Individual Beets of Six Open-pollinated Varieties in mg. CO2 Per kg. Per Hour and Analysis of Variance Table.

	Beet Number										
Variety	1	2	3	4	5	6	7	8	9	10	Mean
C455	284	229	243	279	311	241	392	325	302	369	297.5
GW305	329	311	358	291	321	272	329	336	266	346	314.8
GW304	293	275	242	329	302	302	391	352	327	362	317.5
C359	305	284	254	311	256	199	352	286	256	249	281.2
C478	359	334	291	396	350	320	403	314	284	314	336.5
GW381	284	256	351	275	307	332	282	401	291	406	318.5

Source of Variation	D.F.	Mean square	S.E.	F	F req. for sign 5%	1%
Between varieties	5	3,667		1.8	2.4	3.4
Error	54	1,994	45 (C.V. = 14)			

Shown in Table 1 are some of the differences obtained between duplicate samples of the same beet, between beets of the same variety and between varieties. Variety GW381 was outstandingly higher in respiration rate when compared with Variety GW305. This is an interesting point, however: It was found in subsequent studies that the mean respiration rate of five or even 10 beets of an open-pollinated variety does not represent the population mean. That variation does exist between certain varieties was suggested from results obtained by another method previously reported (3)[2].

In Tables 2 and 3 respiration rates and analysis of variance tables are

[2] Numbers in parentheses refer to literature cited.

given for ten beets of six open-pollinated varieties and six inbred lines, respectively.

Tables 2 and 3 bring out some items of interest with regard to respiration rates. If it is assumed that inbreds listed in Table 3 are stable with regard to all characters (which may be a false assumption) the variations in respiration rates obtained within an inbred must be attributed to environment as it affects condition of roots and other factors which combine to make the error. Based on the foregoing assumption and the statistical analysis given, the only justification for selection would be between inbred lines. A guide to effectiveness of selection in open-pollinated varieties will have to await testing of progeny.

Table 3.—Respiration Rates at 25° C. on Sliced Tissue from Ten Individual Beets of Six Inbred Lines in mg. CO_2 Per kg Per Hour and Analysis of Variance Table.

	Beet Number										
Inbred	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
1,035	355	345	313	525	402	459	441	371	377	310	389.2
1,060	255	335	302	345	289	310	332	370	389	334	326.1
1,036	264	361	276	306	277	268	290	259	261	311	277.3
1,061	233	311	318	250	293	268	272	261	311	264	278.1
1,063	318	251	326	272	350	224	366	329	350	245	303.1
1,051	291	293	358	334	355	297	311	336	321	300	319.6

Source of Variation	**D.F.**	**Mean square**	**S.E.**	**F**	**F req. for sign 5%**	**1%**
Between inbreds	5	17,145		9.7	2.4	3.4
Error	54	1,763	42 (C.V. = 13)			

Method, Results and Discussion of Testing Individual Roots for Storage Rot Resistance

Method—The method of testing individual roots for storage rot resistance is an adaptation of the method developed by Gaskill (2). The method makes possible the testing for storage rot resistance in conjunction with assembly line testing for other characters.

After the beet is rasped, a 20-30° sector of tissue is sawed out just adjacent to the rasped sector. This is accomplished by passing the beet, in a rasping cup, over a circular saw. The sector is lifted out and a two-inch piece (Figure 1) is saved from the shoulder of the beet. A small tab bearing the beet number is pinned to the slice for identification.

In tests conducted in 1950-51, two areas on the epidermis of the sector, one-fourth inch in diameter, were pared off with a vegetable peeler just prior to inoculation. This method did not give 100 percent infection at the point of inoculation. Consequently, in 1951-52 the apex of the sector was sliced off and discarded and the inoculum applied to the freshly cut surface of the sector saved.

The inoculum was prepared from a pure culture of a proven pathogenic strain of *Phoma betae.* Cultures grown in three Petri plates were mixed wtih 125 cc. of water agar and diluted with an equal volume of water

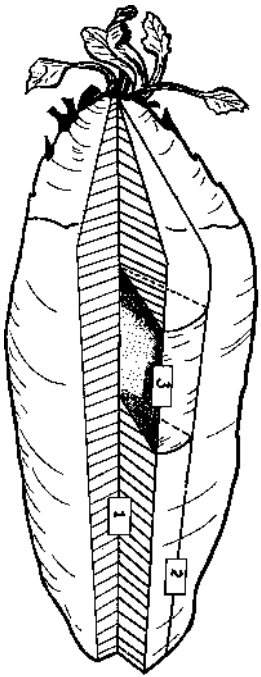

Figure 1.—Sketch o£ beet showing (1) rasped sector, (2) saw cut and (3) portion of root which is removed for inoculation.

to provide inoculum of ideal consistency for dropping. A drop of inoculum was placed on each pared area in 1950-51 and toward each end of the newly exposed area in 1951-52. A 24- x 30-inch tray with a one-quarter inch mesh screen bottom was used to hold the sectors. Each tray held approximately 200 inoculated pieces. An insulated waterproof cabinet with a capacity of 45 trays was built for incubating the inoculated beet sectors. Heat was controlled by a thermoregulator and supplied by three 100-watt incandescent light bulbs. A saturated humidity was maintained through the use of a medicinal atomizer attached to an air compressor and a supply of water.

The inoculated pieces were incubated for four weeks at 45° F. in 1950-51, and for 11 days at 55° F. in 1951-52. After the period of incubation, the inoculated sectors were split longitudinally through the two spots on which the inoculum was applied. The depth of penetration of the rot from the

surface was measured in millimeters at the point of inoculation. This measurement became the criterion on which susceptibility or resistance was established.

Results and Discussion—An attempt was made in 1950-51 to improve a commercial variety in storage rot resistance, among other characters, by mass selection. Pieces of 2,000 roots grown at McCook, Nebraska, were inoculated. After four weeks' incubation few pieces showed any signs of storage rot. For this reason a positive selection could not be made. Beet slices from inbred lines grown at Longmont, Colorado, however, rotted sufficiently so that between line differentiation could be made. The within line variation, as to rot penetration, was somewhat erratic.

The penetration of rots in 1951-52 obtained by inoculating the inner tissue of the beet appeared to be much more consistent than the depth of penetration obtained in 1950-51. Unmistakable differences between inbred lines are shown in Table 4.

Table 4.—The Distribution of Roots Per Class as to Depth of Penetration in Sectors of Roots of Inbred Lines and an Open-pollinated Variety Inoculated with *Phoma Betae* and Incubated at 55° F. for 11 Days, 1951-52.

Plot No.	Rot Penetration (mm)										Mean Rot Penetration mm	Mean Sugar Percent
	0-1	1½/2	2½/3	3½/4	5	6	7	8	9	10		
2,221	10										1.1	14.0
2,223	1	3	5	1							2.6	12.9
2,255[1]	1	3		1							2.1	13.9
2,248				2		2	1	2	1	1	7.8	10.2
2,252[1]	1	1	2	1							2.4	13.1
2,253	10										0.6	13.8
2,271A[2]	4	5	2	1							1.7	15.8
2,272A[3]	2	5	2	1							2.0	14.3

[1] Open-pollinated variety.
[2] Inbred line grown in different part of field from plots 2,221-2,253.
[3] Open-pollinated variety grown adjacent to inbred line in plot 2,571A.

The differences are also very vividly illustrated in Figure 2.

Inbred lines included on the table may be considered genetically stable as they have resulted from selfing four or more generations.

The results obtained leave no doubt that inbred lines grown under environmental conditions which favor rotting can be differentiated as to degree of resistance by this method.

Another question arises, however: Is the measure of resistance obtained by this method anything more than a measure of sugar content? A rather close relationship between high sugar content and storage rot resistance was observed and is illustrated in Table 4. Inbred lines in Plots 2,221 through 2,253 were grown with a relative lack of competition which resulted in a lowered percentage of sugar. The inbred line in Plot 2,248 which had an extremely low percentage of sugar was susceptible to storage rot and lines in plots 2,221 and 2,253, which had a relatively high percentage of sugar, were resistant to storage rot.

Figure 2.—Root slices of inbred lines after inoculation with *Phoma betae* and incubation for 11 days at 55° F. Left to right—Plot 2,221 resistant, Plot 2,223 moderately resistant and Plot 2,248 very susceptible.

Storage rot tests made on beets from the same field but under normal competitive conditions, which resulted in high percentage of sugar, failed to reveal the consistency of rot penetration which was obtained in the low sugar portion of the field. Considering the variation in readings between beets within Plots 2,517A and Plots 2,572A, the comparisons should probably be confined to between lines and made only with a large number of roots per line.

Additional information is unquestionably needed before any testing method can be said to give definite results in improvement of storage rot resistance, *per se.* Suggested lines of research would include the determination of (a) statistical correlation coefficients of sugar on storage rot resistance, (b) optimum incubation time, (c) the correct organism or group of organisms to use, and (d) optimum temperature for incubation.

Some pertinent research has been done by Russian workers (1) (4) in which *Botrytis cinerea* was used for inoculation. The incubation was conducted at 77° Fahrenheit for five or six days. Stock beets which were low in percentage of sugar were reported as most susceptible. On the other hand, Gaskill (2) reports that red garden beets which are low in percentage of sugar were the most resistant of the varieties of beets which he tested.

Summary

Methods for comparing respiration rates and storage rot resistance of individual beet roots are described. The methods are rapid and are sufficiently accurate to make what appear valid selections between inbred lines on **which means have been drawn from determinations of several beets of** the **line. Selection on basis of individual root analysis of open-pollinated varieties remain of doubtful merit until proven or disproven by tests of progeny.**

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