A Method for Determining Respiration Rate and Sampling for Chemical Analysis of Individual Sugar Beets

MYRON STOUT

Introduction

Improvements in methods of handling and storing sugar beets have been rapid during the past decade. The mechanical harvester has brought about direct delivery of the crop after lifting, thereby eliminating exposure of the beets to frost, sun and wind before piling. This, plus greater use of forced ventilation of storage piles, has done much to reduce storage losses by providing a better storage environment within the pile. Further im-provement seems to be divided into two separate approaches: 1. improve-ments in harvester and piler efficiency and more effective control of the environment within the pile and on its surface exposures; 2. improvement of the beet itself, to reduce respiration rate and susceptibility to attack by fungi.

The first approach can be best accomplished by engineers, machinery manufacturers and agricultural departments of the sugar companies who design, make and use or supervise the use of the equipment and have direct control over the large and expensive installations.

The second approach of improving the beet itself has been studied very little in modern breeding methods. The old method, used by all sugar-beet breeders, of selecting superior mother beets or stecklings in the fall, storing until the following spring and replanting them for seed pro-duction, has eliminated most lines or individuals that were extremely sus-ceptible to attack by fungi. This has greatly improved the sugar beet for resistance to spoilage, but individual differences are still evident within com-mercial varieties and some inbred lines are extremely susceptible to spoil-age. Gaskill (l)[2] has shown by progeny tests that greater resistance to attack by certain fungi can be bred into lines of beets.

Very little work has been done to breed beets for low respiration rate. This has been partly due to the lack of a rapid method of sampling and conducting respiration rate measurements, as well as chemical analyses on an individual beet basis without impairing the subsequent growth of the beet. Nelson and Oldemeyer (2) reported respiration rate studies on sliced pieces of uniform thickness from the tail section of the beet. Res-piration rates of these slices, ranged from about 150 to 300 mg. of CO_2 per kg. of beets per hour at 20° C. The present report describes a sampling technique, a method of measuring respiration rate, and preparation of diffusate for chemical analysis without undue injury of the beet for sub-sequent growth. This method is patterned somewhat after that described by Nelson and Oldemeyer, except that the sample is in the form of a cylinder. The respiration rates on the samples here reported are only about 1/3 that of the sliced tissue samples used by Nelson and Oldemeyer due to the reduced surface area of the samples.

[1] Physiologist, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

[2] Numbers in parentheses refer to literature cited.

The wide variations between individual beets within commercial var-ieties probably result from both heredity and cultural environment. Most commercial varieties can be considered as a mixture of related types because of the strong tendency of the beet to cross fertilize in the seed field. Sugar beets are also highly competitive plants with regard to space allotted in the field. A small delay in germination or early development causes a large difference in growth. The magnitude of these differences, both genetic and environmental, can be seen by the mean average values of twenty-five beets of each of twelve varieties grown on plots of equal size under normal competitive environment. Most beets smaller than 600 grams were discarded. Average values for all varieties followed by the average percentage difference or variation between the highest and lowest individuals of each variety were observed to be: weight, 1050 grams, variation 224 percent; sugar percentage 14.22, variation 61 percent; respiration rate 73.3 mg. CO_2/kg/hr., variation 83 percent; amino nitrogen as glutamine percent 0.42, variation 553 percent; sodium 800 ppm. variation 308 percent and potassium 4313 ppm., variation 81 percent. Because of these wide variations, effective selection within a heterogenous population is possible, but it requires that a large number of individuals, grown under normal field competition, be tested and only the best saved for further breeding work. Progeny tests made possible by utilizing special self-fertile types of beets permit still further genetic control.

Sampling Equipment and Technique

The most rapid and convenient test for quality of sugar beets is that for soluble dry substance by means of the Abbe refractometer. The usual procedure has been to drill a hole in the beet and press a drop of juice from the macerated tissue through muslin cloth on the prism of the re-fractometer. The dry substance percentage is read directly on the special scale of the instrument.

A new conically shaped tip, shown in figure 1, allows the juice to be pressed from the macerated tissue while still within the beet and caught in a plastic spoon for transfer to the refractometer prism. The tip does not penetrate as deeply as the drills which have been previously used, no cloth filter is necessary and sampling requires much less time. After a little experience, six people have tested more than 250 beets per hour. This included cleaning one side of the beet, sampling, reading dry substance and recording the values on both beet and paper, as well as numbering and selecting the choice beets. Cleaning the beets proved to be the bottle-neck of the operation. If the beets are washed previously, the testing can be speeded up with one less person, the laboratory is kept cleaner, and there is less damage to the refractometer prism. Probably 50 to 70 percent of the beets of poorer quality should be eliminated by this rapid and simple test. The remaining beets are saved for further tests. From each of the selected beets, a smooth, cylindrical piece of tissue is then cut, by means of the apparatus shown in figure 2, by placing the small hole in the beet made by the dry substance sampling tip, over a small faucet washer on the rubber anvil and forcing the handle down and quickly withdrawing it. An adjustable stop is provided for the handle to prevent the cutting tube from being damaged by severe contact with the rubber anvil. The stationary guide inside the cutter tube guides the cutter tube through the

beet and also forces the cut cylinder from the tube as the handle is raised. The cut cylinder is then easily pushed from the beet.

A piece of tissue, slightly in excess of 10 grams, is cut from the cylinder of tissue by the double-bladed instrument shown in figure 2. The remainder

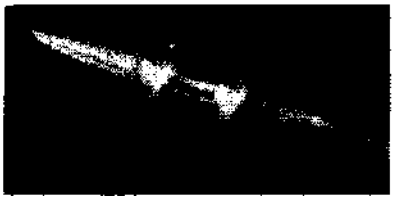

Figure 1.—Improved juice extractor for use with Abbe refractometer. A 1/4-inch brass brazing rod made cone-shaped and split with hack saw. Two washers forced on as shown and a piece of hack saw blade soldered in place. The blade is ground slightly lower on the "cutting side" to insure maceration rather than cutting of tissue. Second washer prevents juice draining into chuck of drill. The drill should be operated in a tilted position at about 200 to 400 r.p.m. The beet is drilled to within about 1/4-inch of the first washer; then with the drill stopped further pressure expresses the juice which is caught in a plastic spoon.

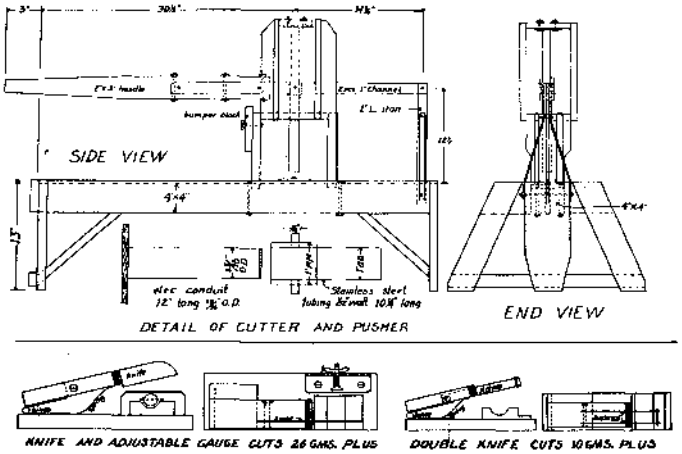

Figure 2.—Apparatus to cut cylinder of tissue from beet. The cutting tube is forced through the beet and quickly withdrawn. This gives a re-markably clean cylinder of tissue with little effort. (See text for details).

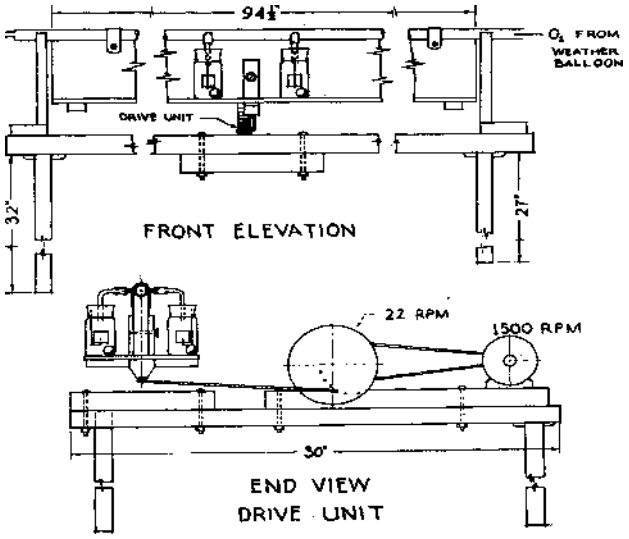

Figure 3.—Respiration apparatus. (See text for details)

of the cylinder can be placed in the groove of the single knife instrument, shown in figure 2, and a piece slightly more than 26 grams cut off. The two pieces of tissue are lightly impaled on stainless steel wires protruding from trays which hold the samples in numerical order. Three people can weigh, number, record, and sample 56 beets in about 30 minutes. The two samples are protected from undue loss of moisture until they are adjusted to weight for respiration rate measurement or disintegration for chemical analysis, as described later.

The cylinders of tissue may also be used for study of the vascular ring structure of the beets.

Measurement of Respiration Rate

The samples cut by the double-bladed knife are adjusted to 10 grams and lightly impaled on stainless steel wires soldered to a stainless steel tray, and washed with water at 45° C. (3 changes of water during 3 min-utes) . They are then washed in water at the temperature of the respiration measurement (usually 20° C.) for 15 minutes, blotted with a towel and stored in a closed humid chamber for three hours before the respiration test is started. (The periods and temperatures given are probably not critical but have given good results. Any procedure adopted should be con-sistently followed.)

The respiration apparatus shown in figure 3 has 58 units. Two units are reserved for checks (without tissue) . Each unit consists of a wide-mouthed bottle 3 1/2 inches high by 2 inches, outside diameter, closed with a No. 9 one-hole rubber stopper. The .stopper is fitted with a piece of bent stainless steel wire to hold the tissue, and is connected to the oxygen mani-fold through semi-capillary glass tubing, rubber tubing, and a small piece of copper tubing soldered into the 1/2-inch pipe. The oxygen manifold is fastened to a one-inch by 4 1/2-inch board that extends the full length of the tilting platform. The base of the tilting platform is made of 1/8-inch masonite and extends 3 1/2 inches on each side of the center supporting board. The base is reinforced with 1/2 x 1 1/2 inch slats spaced about 2 feet apart. The tilting platform is supported by the oxygen manifold pipe that fits into saddle bearings at each end of the tilting platform. The lever arm that operates the tilting platform extends below its base and is operated by a 1/8-inch steel rod connected to a wooden wheel on a small speed-reducing gearbox. The gearbox is v-belt driven by a small electric motor (about 1/10 h.p.) . The gearbox and motor assembly is bolted to the supporting platform through slotted holes to provide for easy adjustment to obtain uniform tilting of the platform. Adjustment of the amplitude of tilt is made by having a series of holes in the wooden wheel on the gearbox through which the connecting rod is inserted. The unit described is oper-ated at about 22 cycles per-minute. This rate can be varied by changing pulleys on the gearbox and motor.

The tilting platform is about 94 1/2 inches long and about 5 inches higher on one end than the other to prevent "dead centering" of the marbles on one side of the bottles. Marbles 3/4 inch in diameter keep rolling much better than smaller ones.

In operation, several tissue samples are placed on the stainless steel-L-shaped supports that extend through the rubber stoppers, 5 ml. of .35 *N* Ba $(OH)_2$ are added to each bottle containing a marble and immedi-ately fitted to the rubber stopper so that the connection is tight and the bottle rests level on the tilting platform. After all samples are in place, a weather balloon, loosely filled with pure oxygen, is connected to the manifold. The opposite end of the manifold is left open. A lighted match or cigarette held by the open end indicates when all the air is swept from the manifold. The manifold is closed and the operation started. Twenty hours after the first samples are placed in position, the apparatus is stopped and the excess Ba $(OH)_2$ in each bottle is titrated with 0.11 *N* HC1 using phenolphthalein indicator. The respiration rate is then calculated by simply subtracting the ml. of HC1 used for each sample from the ml. of HC1 used for the "check" samples (containing no tissue) and multiplying by ten. The respiration rate is recorded as milligrams CO_2 per kilogram of beets per hour. These values are about 5.5 times the rate of respiration of whole beets averaging 1 kg. each.

Duplicate samples taken from opposite sides of the same beets and treated according to the method outlined occasionally vary as much as 10 percent. Unwashed samples were less consistent. Correlation coefficients between duplicate washed samples usually ranged between r = .85 and

r = .95. Unwashed samples sometimes gave values as low as r = .70. Respiratory enzymes in the cut surfaces or sugar that might interfere with gaseous exchange in the surface, may be responsible for the less consistent results with unwashed samples.

Chemical Analysis

A weighed 26-gram piece of the cut cylinder of tissue is sliced into a Waring Blendor equipped with well-sharpened knives, 177 ml. of distilled water added, and the machine run for only one minute and the mixture poured into a monel metal sugar cup. About 0.7 grams of Dr. Home's dry lead acetate (dibasic) is added and the mixture shaken. After about 30 minutes, the sample is again shaken and filtered. About 18 ml. of the filtrate is saved in a plastic-stoppered, 20-ml. bottle for amino N, sodium and potassium determinations and the remainder is used for sugar determination by polarization.

Amino Nitrogen as Glutamine

Ten ml. of the clear filtrate is pipetted into a 25-ml. test tube and 1 ml. of Stanek-Pavlas (3) reagent (10 gms. Cu $(NO_3)_2.3H_20$ plus 250 gms. $NaC_2H_3O_2.3H_2O$ per liter) added. (Better results were obtained at high nitrogen concentrations by increasing the Cu $(NO_3)_2.3H_2O$ to 15 grams per liter.) The solutions are mixed by inverting two or three times and the percent transmittance in a standard 10-mm cell at 620mμ determined with a spectrophotometer or photo-colorimeter, using distilled water as a 100 percent transmittance reference standard. The "amino nitrogen as glutamine" percent on beets is read from a decade table, prepared from a series of standard glutamine solutions (glutamic acid can be used as a standard but is less soluble, although less expensive) . Any lead carbonate that precipitates and clouds the filtrate may result in high values and should be guarded against. For this reason, all sample bottles were well filled and capped to reduce contamination with atmospheric CO_2. The determinations should be run promptly after filtration for the same reason.

Although the method outlined is not as accurate as the Kjeldahl method, it is very fast and well correlated with results for total nitrogen and soluble, non-protein nitrogen in the diffusate from fresh beets, as determined by the Kjeldahl method.

Correlation coefficients calculated on more than thirty fresh-beet samples determined by three methods *in* 1943, gave calculated rvalues as follows:

Total N %. vs. amino N as glutamine %, r = + .967 Total N %. vs. soluble non-protein N %, r = + .990 Soluble non-protein N % vs. amino N as glutamine %. r = +.970

The samples analyzed varied in nitrogen content as follows:

Total N (Kjeldahl)	0.105% to 0.256%.
Soluble non-protein nitrogen (Kjeldahl)	0.045% to 0.188%
Amino N as glutamine (colorimetric)	0.07% to 0.65%.

The method is probably useful to indicate the relative nitrogen content of fresh beets *in* routine or selection work.

Sodium and Potassium

Sodium and potassium are very rapidly determined on the remainder of the sample in the 20-ml. vial by means of the flame spectrophotometer. By coating the small 5 ml. sample cups with Beckman "Desicote," practically all of the previous sample can be removed by shaking; thus speeding up the determinations.

The conversion of all spectrophotometer transmission readings to percentage or parts per million values is greatly facilitated by preparing decade tables for each kind of determination.

Decade tables may be prepared as follows: The concentration for each percentage point of transmission is carefully read from the standardization curve plotted on log log paper. This curve should be nearly a straight line. These values are then calculated to the equivalent parts per million or percentage on beets, taking into consideration the dilution factor used in making the diffusate. If half-normal diffusate is used, the factor for p.p.m. on beets will be 200/26 times the concentration of the standardizing solution. These expanded values will contain larger errors due to the expansion of the values as read from the curves, especially at the higher concentrations. Errors in reading the standard curve may be reduced by making about three overlapping curves on the same sheet of log log paper. If the log scale is repeated on each axis, one curve can represent from 10 to 1,000 p.p.m., the second from 1 to 100 p.p.m. and the third from .1 to about 7 p.p.m. in the standard solutions. Errors in the expanded values may be reduced by plotting a limited part of the data at a time on large sheets of linear cross-section paper. Values for tenths or two-tenths increments of transmission readings are filled in linearly between successive percentage transmission values.

With the aid of such a table, the transmission values can be converted to p.p.m. or percent on beets very rapidly. A standard solution should be run every five or ten determinations to insure that the atomizer burner is feeding at a constant rate.

If the transmission reading of the standard solution is in error more than one or two percent, the capillary tube of the atomizer burner should be cleaned. Slight errors may be corrected by adjusting the slit width and re-running the samples back to the previous standard.

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