# USE OF EXPRESSED SAP IN PHYSIOLOGIC STUDIES OF CORN[1]

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(WITH FOUR FIGURES)

In planning an extensive series of physiologic studies with corn, the need was felt for rapid, simple, yet accurate methods of making certain determinations. Plant physiologists have been using sap expressed under high pressures in various physiologic studies for many years. The apparatus and methods used and the results obtained have differed so widely that it was considered desirable to try different methods to find one that could be used satisfactorily in the investigations in mind. A description of the apparatus and procedure found satisfactory in obtaining samples of sap from vegetative parts of the corn plant suitable for some phases of the investigations, and data showing some of the limits within which such samples may be used are presented in this paper.

## Apparatus and methods

A small laboratory hydraulic press (fig. 1) was used for expressing the sap from the corn tissues. A cage to contain the tissues, similar to that described by MEYER (**2**), was constructed so that the 25,000 pounds total working load exerted a pressure of 5,000 pounds per square inch on the tissue in the cage. The apparatus was arranged so that the sap obtained was filtered. The filter consisted of a layer of linen cloth and a layer of 100-mesh copper sieve wire placed over a perforated steel disk in the bottom of the cage. The disk was perforated with one-sixteenth inch holes, arranged radially in rows, with grooves on the lower side of the disk for drainage. Filtered through this, the sap contained no cell residue and was practically free from suspended material. The cage was sealed liquid tight by a rubber stopper placed above the tissue.

A uniform time of draining was found necessary for obtaining comparable results. If many samples are to be examined this time must be a minimum. In these experiments five minutes were allowed for draining, during which time the pressure was maintained at 25,000 pounds; this was long enough to express practically all of the obtainable sap.

In some of the experiments it was desired to test successive portions of the sap. The successive 10-cc. samples of sap from 100 grams of tissue were collected in separate 10-cc. graduated cylinders. The same process

[1] Based on investigations cooperative between the Ohio State Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

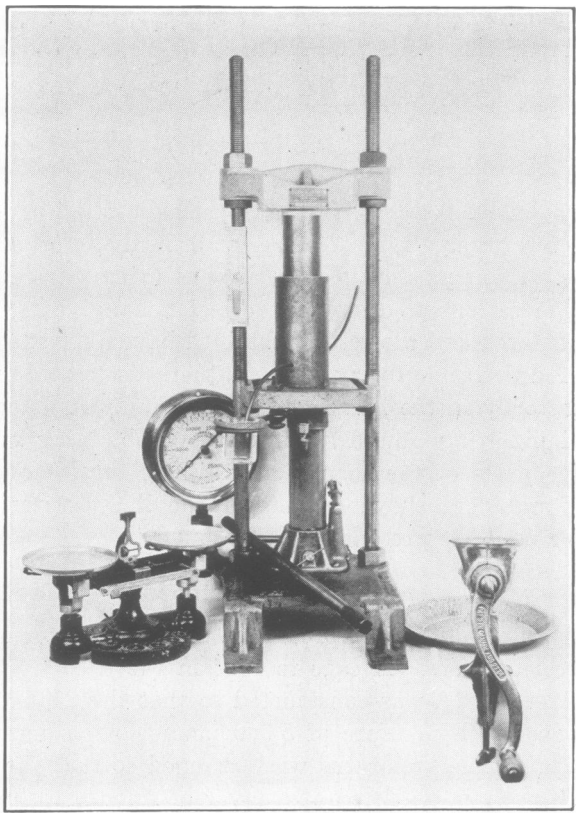

FIG. 1. Equipment used for obtaining samples of expressed sap from corn tissue.

was repeated with two other 100-gram samples of tissue and the corresponding portions from the three expressions were mixed to provide samples for examination.

Total solids in the sap were determined with the refractometer, GORTNER and HOFFMAN (**1**). The total reducing power of the sap was determined by a modification of the QUISUMBING-THOMAS method (**3**) after inversion of the sucrose by invertase. In analyzing the plant tissue for sugars for comparison with estimates based on the sap analyses the samples, which had been preserved in alcohol, were extracted with 80 per cent. alcohol and the total reducing power determined as above. In the comparisons shown this is designated as the "standard" method in contrast to the "sap" method.

## Results

The experiments fall naturally into studies of (1) factors involving the amount of sap or water expressed, (2) the sugars and total solids in the sap

and in successive portions thereof, and (3) the relation between the sugars in the sap and in the tissue. The results with sucrose and with free reducing sugars were so alike that they have been combined, and only total sugars are reported.

## THE AMOUNT OF SAP EXPRESSED

Among the factors affecting the amount of sap which could be expressed studies were made of the preliminary treatment of the tissue, the size of sample, and the kind of tissue and its moisture content.

*Effects of preliminary treatment.*—Various methods of treating the tissue prior to expressing the sap have been used by other workers. These methods have been summarized by MEYER (**2**). Here, preparation by grinding the tissue with a food grinder (fig. 1) and mincing it, with and without freezing it to –9° C. in each case were compared. The grams of sap recovered from 100 grams of blade tissue of Burr-Leaming corn and of orchard grass after the different treatments are shown in table I.

TABLE I

THE AMOUNT OF SAP EXPRESSED FROM BLADE TISSUE OF CORN AND OF ORCHARD GRASS FOLLOWING DIFFERENT TREATMENTS
(AVERAGE OF THREE DETERMINATIONS)

| BLADE TISSUE FROM | AMOUNT OF SAP FROM 100 GRAMS OF TISSUE | | | |
|---|---|---|---|---|
| | GROUND | GROUND AND FROZEN | MINCED | MINCED AND FROZEN |
| | *gm.* | *gm.* | *gm.* | *gm.* |
| Corn | 71 | 72 | 26 | 45 |
| Orchard grass | 53 | 58 | 13 | 33 |

More sap was recovered after grinding only than after mincing, either with or without freezing. Moreover, the additional amount of sap recovered after grinding and freezing the tissue, above that obtained after grinding only, was negligible. Grinding is more simple and rapid, and omission of freezing avoids any danger of change in the colloids that might result from that process. Finally, as will be shown later, successive portions of sap were more nearly alike after grinding than after mincing. Grinding seemed to be the preferable method of preparation and accordingly was used in the succeeding experiments reported in this paper.

THE INFLUENCE OF SIZE OF SAMPLE.—Using uniform pressures and times of draining, samples of 50, 100, and 150 grams were compared. Representative data for different tissues are given in table II.

TABLE II

PERCENTAGE OF TOTAL WEIGHT EXPRESSED AS SAP FROM DIFFERENT SIZED SAMPLES OF DIFFERENT CORN TISSUES

| KIND OF TISSUE | PERCENTAGE TOTAL WEIGHT EXPRESSED FROM SAMPLES OF: | | |
|---|---|---|---|
| | 50 GRAMS | 100 GRAMS | 150 GRAMS |
| | *per cent.* | *per cent.* | *per cent.* |
| Stem | 82 | 80 | 80 |
| Blade | 78 | 72 | 66 |

Larger percentages of sap were removed from the smaller samples, particularly of the blade tissue. As will be shown later, the sap remaining in the press cake is similar in composition, in certain respects, to that expressed. Accordingly, the 100-gram sample was used in our experiments in order to provide adequate quantities of sap and moderately high expression. For some purposes it probably would be better to obtain the necessary sap from two smaller samples.

KIND OF TISSUE AND MOISTURE CONTENT.—The amount of sap removed by pressure from blade and stem tissue of Burr-Leaming corn at different stages in its development is shown graphically in figure 2. The amount of sap obtained has been expressed as cubic centimeters of water (by subtracting the total solids from the weight of sap obtained) for comparison with the absolute moisture content of the tissue.

The amount of water which was expressed naturally was highly correlated with the amount in the tissue. This correlation was not perfect, however, a larger amount of water remaining in the blade tissue (20 to 27 cc.) than in the stem tissue (8 to 10 cc.). In the blade tissue, moreover, the absolute amount left after pressure extraction was larger in the samples obtained later in the season and in which the original moisture contents were less.

### SOLIDS AND SUGARS IN SUCCESSIVE PORTIONS OF SAP

If successive portions of sap expressed from a given sample of tissue have the same composition with respect to certain constituents, the sap remaining in the press cake may be assumed also to have this composition. The total solids, sucrose, and free reducing sugars were determined in suc-

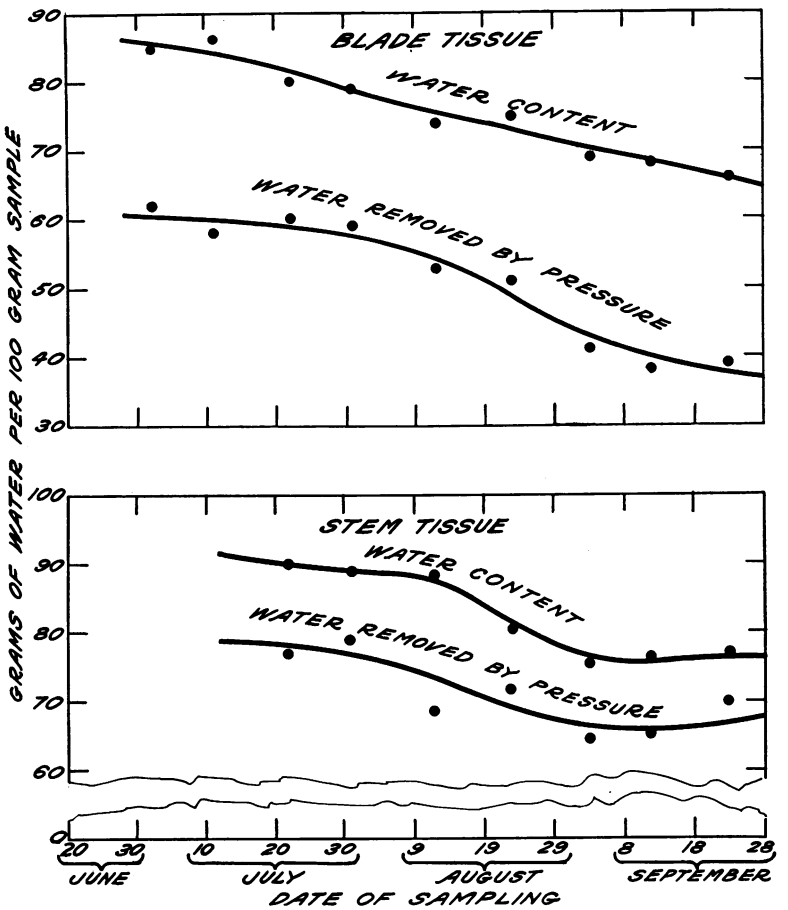


FIG. 2. The water content and the amount of water removed by pressure, from blade and from stem tissue of Burr-Leaming corn, summer 1929.

cessive portions of sap from different tissues after grinding only and after mincing. The data from a representative experiment are shown in table III.

The solids content decreased in the successive portions of sap from all samples. The decrease was greater with blade than with stem tissue, and greater with minced than with ground tissue. The maximum decrease was from 6.2 per cent. of solids in the first portion to 4.2 per cent. in the last portion of sap from minced blade tissue. The corresponding decrease for the ground tissue was from 11.1 to 9.1 per cent. The differences with the stem tissue were similar though usually smaller.

The percentages of sugars were practically the same in the successive portions from ground tissue, whether stem or blade. With the minced

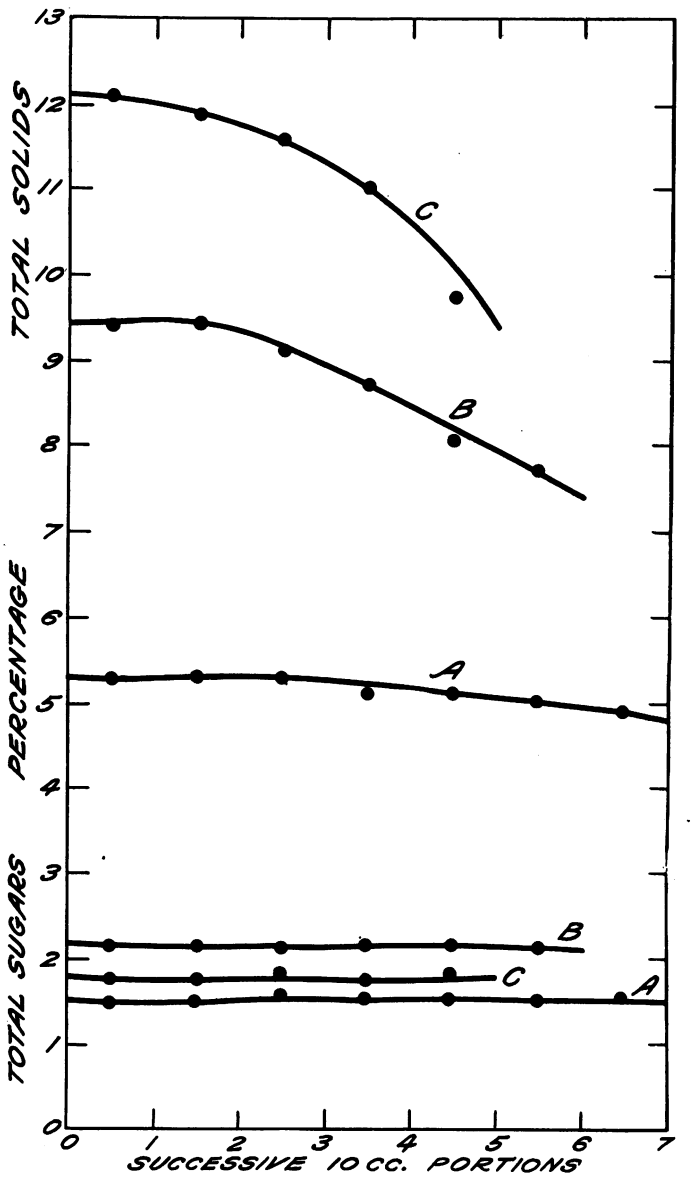


FIG. 3. Percentage of total solids and total sugars in successive 10-cc. portions of sap from blade tissue of Burr-Leaming corn. A = 86 per cent. moisture, B = 74 per cent. moisture, C = 70 per cent. moisture.

## TABLE III

PERCENTAGES OF TOTAL SOLIDS AND TOTAL SUGARS, IN SUCCESSIVE 10-CC. PORTIONS OF EXPRESSED SAP OF DIFFERENT CORN TISSUES AFTER DIFFERENT TREATMENTS

| PORTION | GROUND | | | | MINCED | | | |
|---|---|---|---|---|---|---|---|---|
| | STEM | | BLADE | | STEM | | BLADE | |
| | SOLIDS | SUGARS | SOLIDS | SUGARS | SOLIDS | SUGARS | SOLIDS | SUGARS |
| | *per cent.* | *per cent.* | *per cent.* | *per cent.* | *per cent.* | *per cent.* | *per cent.* | *per cent.* |
| 1 | 12.9 | 12.1 | 11.1 | 2.9 | 9.9 | 10.1 | 6.2 | 2.4 |
| 2 | 12.9 | 12.2 | 11.3 | 2.9 | 9.9 | 10.0 | 4.2 | 1.1 |
| 3 | 12.8 | 12.1 | 10.5 | 2.8 | 9.3 | 9.1 | ..... | ..... |
| 4 | 12.8 | 12.1 | 10.3 | 2.9 | 8.7 | 8.0 | ..... | ..... |
| 5 | 12.8 | 12.3 | 9.6 | 2.7 | 7.9 | 7.5 | ..... | ..... |
| 6 | 12.8 | 12.4 | 9.1 | 2.9 | ..... | ..... | ..... | ..... |
| 7 | 12.2 | 12.2 | ..... | ..... | ..... | ..... | ..... | ..... |
| Last drop | 11.8 | ..... | 8.5 | ..... | 7.5 | ..... | 4.0 | ..... |
| Duplicate 100-gm. sample | 12.9 | 12.1 | 10.4 | 2.9 | 9.4 | 9.3 | 5.2 | 1.8 |

tissues, the sugars decreased in the successive portions similarly to the solids. Grinding the tissue resulted not only in obtaining larger percentages of solids in the sap but also in obtaining sap having the same sugar content in successive portions.

The total solids and sugars in successive portions of sap from stem and from blade tissues of corn after grinding were determined at three different stages of growth during the season. The percentages in the blade tissues, containing 86, 74, and 70 per cent. of moisture respectively, are shown graphically in figure 3.

The total solids decreased with the successive portions, the decrease being the largest for the drier (more advanced) tissue and least for the tissue containing the most moisture. Similar differences, but much smaller ones, were found in the successive portions of sap from stem tissue. The total sugar content was the same in the successive portions of sap for both kinds of tissue at all stages of development.

### RELATION BETWEEN THE SUGAR CONTENT OF SAP AND TISSUE

The sugar content of successive portions of sap expressed from ground tissue was the same for both kinds of tissue and for three stages of development. It seemed probable, therefore, that the sugar content of the tissue could be calculated with more or less accuracy from the sugar content of the expressed sap and the moisture content of the tissue.

During a seasonal study of the distribution of sugars in the corn plant, 58 pairs of samples for sugar determinations were taken. The sugar con-

tent of one sample of each pair was determined by the "standard" alcoholic extraction method and the sugar content of the other was estimated from the sap analysis and the moisture content. In calculating the sugar content of the tissue from sap analysis, no correction was made for the specific gravity and total solids content of the sap. Subsequent data have shown that without this correction the tendency is for the sugar content of the tissue estimated from sap analysis to be slightly low. The samples comprised blade, sheath, and stem tissues from Burr-Leaming and Medina Pride corn, during the period from July 2 to September 24.

The average sugar content of the three tissues from each kind of corn as determined by the two methods is shown in table IV, and the detailed comparison for Burr-Leaming tissues is shown graphically in fig. 4.

TABLE IV

PERCENTAGE SUGAR CONTENT OF CORN TISSUE (1) DETERMINED BY THE STANDARD METHOD, AND (2) CALCULATED FROM THE SUGARS IN EXPRESSED SAP AND THE MOISTURE IN THE TISSUE

| TISSUE | BURR-LEAMING | | | | MEDINA PRIDE | | | |
|---|---|---|---|---|---|---|---|---|
| | NUMBER OF SAMPLES | METHOD | | DIFFERENCE | NUMBER OF SAMPLES | METHOD | | DIFFERENCE |
| | | STANDARD | SAP | | | STANDARD | SAP | |
| | | *per cent.* | *per cent.* | | | | | |
| Blade | 9 | 2.14 | 2.06 | − 0.08 | 9 | 2.11 | 2.26 | + 0.15 |
| Sheath | 17 | 4.79 | 4.50 | − 0.29 | 7 | 4.56 | 4.40 | − 0.16 |
| Stem | 14 | 7.82 | 7.71 | − 0.11 | 12 | 7.98 | 7.42 | − 0.56 |
| All | 30 | 5.41 | 5.27 | − 0.14 | 28 | 5.24 | 5.00 | − 0.24 |
| All, both varieties | 58 | 5.33 | 5.14 | − 0.19 | | | | ... |

The maximum difference between methods was a deficiency of 0.9 per cent. in stem tissue of Medina Pride having a sugar content of 9.1 per cent. (standard method), or a difference of 10 per cent. On the other hand, a sample of blade tissue of the same variety, containing 2.5 per cent. of sugar by the standard method, was estimated as containing 3.2 per cent. by the sap method. The difference here is smaller absolutely but larger relatively, being 28 per cent. of the total. In general, the sugar content indicated by the sap method tended to be lower, the mean difference for the 58 pairs of samples being $-0.19 \pm 0.034$ per cent.

As an additional criterion, duplicate samples of tissue corresponding to 12 of these 58 were analyzed by the standard method and the values compared with the original determinations. These samples comprised different tissues at different stages of growth with a range in sugar content of from 2.68 to 10.50 per cent. The average difference between the paired samples

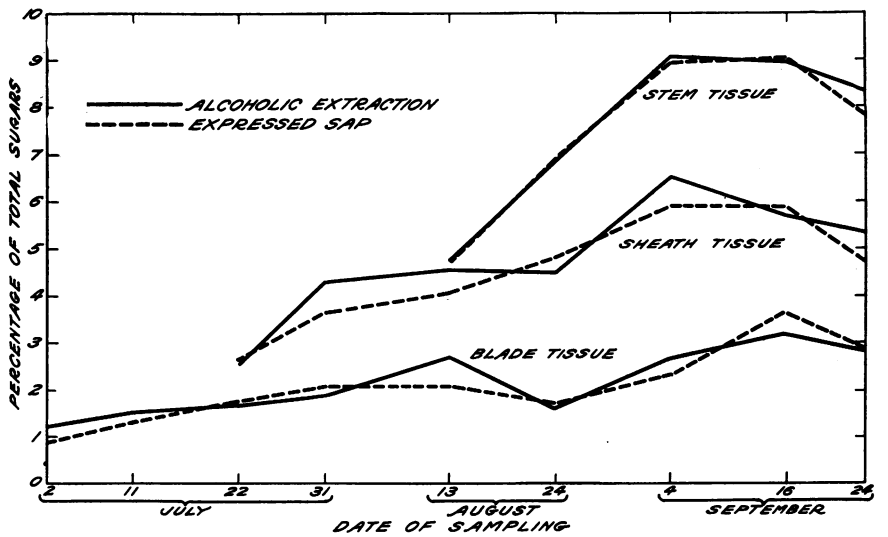


FIG. 4. Percentage of total sugars in blade, sheath, and stem tissue of Burr-Leaming corn obtained by the alcoholic extraction of the tissue and by the expressed sap method.

was $0.26 \pm 0.072$. The results with the two methods accordingly were no more inconsistent for 58 samples than were those by the standard method for 12 samples, and the difference of $-0.19 \pm 0.034$, though significant statistically, must be considered unimportant in a comparison of the two methods.

The agreement between methods over the entire range of the 58 samples is measured best by the coefficient of correlation between the corresponding values. This coefficient is 0.9913, indicating excellent agreement, with only 1.73 per cent. of the variation (as variance) independent. The data indicate, then, that the sugar content of corn tissues may be estimated from the analysis of sap expressed as described, with little or no error in excess of that to be expected from duplicate samples analyzed by the standard method.

## Summary

1. The amount of sap expressed from corn tissue under a uniform pressure and with a uniform time for draining the cage was influenced by the preliminary treatment of the tissue, by the size of sample, and by the kind of tissue and its moisture content as affected by its stage of development.

2. The total solids content differed in successive portions of sap expressed from corn tissue, the size of the differences varying with the treatment preliminary to expression, the kind of tissue and its moisture content.

3. The sucrose and free reducing sugars, reported together here as total sugars, were constant for successive portions of sap expressed after grinding the tissue, but decreased in successive portions from minced tissue.

4. The percentages of total sugars in three kinds of fresh corn tissue from two varieties, at different stages of growth, as determined by the standard method and from the sap expressed after grinding, were in excellent agreement, the coefficient of correlation being 0.9913. The values by the two methods were in as good agreement as those for 12 pairs of samples for which sugars were determined by the standard method.

5. Expressing the sap from a 100-gram sample of ground fresh corn tissue under a pressure of 5,000 pounds per square inch, at the same time forcing the sap through a filter, and allowing a uniform time (5 minutes) for draining, provided samples of sap that were satisfactory for certain physical and chemical examinations.

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