DETERMINATION OF TOTAL AVAILABLE CARBOHYDRATES IN PLANTS

H . W E I N M A N N

The estimation of carbohydrates plays an important part in both pure and applied plant physiology. While satisfactory methods of extracting and determining the individual types of carbohydrates are available, the complete fractionation of these is a tedious process. Furthermore, for many purposes of a more applied nature the determination of the total available carbohydrate content is of greater significance than that of individual carbohydrates or groups of carbohydrates.

The term "total available carbohydrate" may be defined as including all those carbohydrates which can be used in the plant body as a source of energy or as building material, either directly or indirectly after having been broken down by enzymes. In most ordinary, higher green plants the bulk of available carbohydrate is composed of sugars, fructosans, dextrin and starch, whereas hemicelluloses and true cellulose act merely as structural materials and as such cannot further be utilized in the same way as the former (1, 6, 7, 8, 9, 11).

Apparently there is no satisfactory method of assessing the total available carbohydrate content by means of a single determination. Acid hydrolysis of entire samples has occasionally been used as a means of determining total "hydrolyzable" carbohydrate. The results of such determinations, however, are bound to be inaccurate and even erroneous, because it is impossible to separate the starch fraction from the structural carbohydrates in this way, and also because the relatively severe conditions of hydrolysis required for the complete breakdown of starch and dextrin result in the destruction of most of the free and combined fructose (5).

Methods

In the method described below small samples of finely-ground, air-dry plant material are subjected to digestion by takadiastase under such conditions as are necessary for the breakdown of starch, dextrin, and maltose to glucose (4). Other sugars and fructosan are extracted at the same time and, after clarification are converted to reducing sugars by suitable acid hydrolysis; after this procedure the reducing power of the neutralized hydrolysate is determined.

To prevent chemical changes during storage the samples should be killed immediately after harvesting. This can be done by autoclaving for five minutes under five pounds of pressure or, if an autoclave is not available, by some other suitable heating treatment of short duration (5). The killed material is dried in the air, or at a temperature of 40° to 60° C. When air-

dry, the material may be divided into its morphological constituents (stems, leaves, roots, rhizomes) and weighed if required. It is then ground to a fine powder and stored in air-tight bottles.

SOLUTIONS REQUIRED

BUFFER SOLUTION.—A buffer solution of pH 4.45 is prepared by mixing three volume parts of 0.2 N acetic acid with two volume parts of 0.2 N sodium acetate solution. To one liter of this solution one gram of powdered thymol is added. This dissolves slowly but almost completely. The thymol not only preserves the buffer solution indefinitely but also acts as an efficient antiseptic in the digestion of the plant material by the takadiastase (3).

TAKADIASTASE SOLUTION.—Commercial takadiastase contains a large proportion of free-reducing substances, and it is essential to remove these by means of dialysis in order to avoid undesirably high blank values. A suitable membrane may be prepared from a 4% pyroxylin solution in the following way: four grams of pyroxylin are soaked for 15 minutes in 25 ml. of absolute alcohol to which 75 ml. of ether are then added, the mixture is stirred and left standing in a closed vessel until the solution becomes clear. A large Soxhlet extraction thimble is partly filled with the solution and drained so that the liquid forms a continuous membrane on the inside of the thimble. The membrane is dried in a moderately warm place for from three to five minutes; i.e., until most of the ether has evaporated. The degree of evaporation can easily be judged by the odor. During drying the thimble is constantly rotated and occasionally blown into. The thimble with the membrane is kept in water until used.

For the dialysis five grams of commercial takadiastase are dissolved in a convenient volume of water. The solution is transferred to the thimble which is kept suspended in a jar of running tap water for three or four days. At the end of this period the dialyzed solution is filtered into a one-liter standard flask, and made to volume. Dialyzed takadiastase solutions, prepared in this way, were found to be as active as the original material while containing only traces of reducing substances. Stored in the dark, preferably in a refrigerator and with the addition of a small amount of thymol, the solution will retain its activity for approximately six weeks.

If "undiluted" takadiastase of little or no reducing power is available, its use would make such dialysis treatment unnecessary; a 0.5% solution should be satisfactory for the purpose.

PROCEDURE

Weigh out a 0.1- to 1-gram sample of air-dry material and transfer to a 100-ml. Erlenmeyer flask which has previously been weighed accurately to the nearest 0.01 gram. The weight of sample to be used depends on the total available carbohydrate content of the material (see below under section "substrate-enzyme ratio"). Add 10 ml. of distilled water and heat for ½ hour on the boiling water-bath to gelatinize starch, inserting a small glass

funnel into the neck of the flask to minimize the loss of water. Cool to room temperature, wipe the moisture from the outside of the flask and the inside of its neck with a clean rag, place the flask on a balance, and by means of a dropping bottle add as many drops of distilled water as necessary to replace the water lost by evaporation. The counterpoise to be used must equal the weight of the flask plus the weight of the air-dry sample plus 10.0 grams. Pipette 10 ml. of buffer solution and 10 ml. of takadiastase solution into the flask, thus increasing the liquid volume of the digest to 30.0 ml. The moisture content of the air-dry sample will usually be negligible but may be taken into account if considered necessary. Stopper the flask tightly with a well-fitting rubber stopper, and incubate for 44 hours at 37° to 38° C., shaking the flask occasionally.

Cool to room temperature, add 50 to 100 mg. of powdered neutral lead acetate, shake, and allow precipitate and residue to settle. Test for completeness of the reaction with a single drop of dilute potassium oxalate solution, and filter, without washing, through a dry, highly retentive filter (such as Whatman no. 42) into a dry flask containing 100 to 200 mg. of powdered potassium oxalate. Shake, test for completeness of deleading with a drop of dilute lead acetate solution, stopper, and let stand from three to four hours, or overnight in a refrigerator.

Filter and hydrolyze a 15-ml. aliquot with 0.75 ml. of 25% hydrochloric acid for ½ hour on the boiling water bath, attaching the flask to a reflux condenser. Cool, transfer quantitatively to a 50-ml. standard flask, nearly neutralize with 25% sodium hydroxide solution (using a few drops of methyl-red solution as indicator), and make to volume. The final extract volume (50 ml.) contains 15/30 (one-half) the total available carbohydrate of the original digest.

The reducing power of the extract may be determined by any suitable reducing sugar method. In the present experiments a previously described semimicro method using 5-ml. aliquots was employed for all determinations (10). Blank determinations should be carried out whenever a new takadiastase solution is used. For this purpose, pipette 10 ml. of distilled water, 10 ml. of buffer solution, and 10 ml. of the takadiastase solution into a 100-ml. Erlenmeyer flask. Incubate, and subsequently treat the blank digest in exactly the same way as the other digests (except for the gelatinization treatment which is omitted in the case of the blanks). Subtract the blank titration value from the titration value for the plant digest, calculate as glucose, and report as "total available carbohydrate."

A considerable number of determinations can be completed with this method in a relatively short time. The necessary equipment being available, a single worker can carry out 12 determinations simultaneously. If on each of the first three days one set of 12 determinations is started, these can be completed in succession during the following three days, making 36 determinations per week.

It is tentatively suggested that the method may also be of interest to students of problems of human nutrition. The carbohydrates determined by the described procedure are essentially those constituting that carbohydrate fraction in vegetable foods which is digestible by the human organism. The direct estimation of this fraction may well be superior to its calculation by subtraction, as is done in the conventional analysis of foodstuffs.

Experimental data

DIGESTIBILITY OF VARIOUS CARBOHYDRATES

The conditions under which takadiastase converts starch completely to glucose have been ascertained by DENNY (4). YEMM (14) used takadiastase successfully for the hydrolysis of maltose as well. A series of preliminary

TABLE I

TAKADIASTASE DIGESTIBILITY OF VARIOUS CARBOHYDRATES

| | GLUCOSE PER DIGEST | | PERCENTAGE DIGESTED BY TAKADIASTASE |
CARBOHYDRATE	BEFORE ACID HYDROLYSIS	AFTER ACID HYDROLYSIS	
	mg.	*mg.*	*%*
Rice starch	25.4	26.6†	95.5
Maize starch (technical)	23.0	23.4†	98.3
Yellow dextrin (Merck's)	13.8	26.2†	52.7
Maltose (Schering-Kahlbaum)	24.5	25.9†	94.5
Dextrin-Maltose mixture*	25.1	26.5†	94.7
Inulin (Gurr)	0.16	24.5‡	0.65
Sucrose (Analar)	19.3	25.6‡	75.4

* Prepared by action of saliva upon rice starch; dextrin: maltose = 1: 4 (approx.).
† Hydrolyzed 20 ml. of the digest with 1.6 ml. 25% HCl for 1 hour at 15 lb. pressure.
‡ Hydrolyzed 20 ml. of the digest with 1.0 ml. 25% HCl for $\frac{1}{4}$ hour on the boiling water bath.

experiments was carried out to test the digestibility of various carbohydrates by takadiastase under the conditions proposed by DENNY (4) and subsequently adopted in the method of estimating total available carbohydrates.

Solutions of starch, dextrin, maltose, sucrose, and a suspension of inulin were prepared. The starch and dextrin solutions were boiled three minutes before being cooled and made to volume. Twenty-five mg. of carbohydrate were used in each digest, the total volume of which was adjusted to 50 ml., including 10 ml. of dialyzed takadiastase solution (corresponding to 50 mg. original takadiastase) and 20 ml. buffer solution (pH 4.45). After an incubation period of 44 hours at 37° to 38° C. the reducing power of the digests was determined and an aliquot was hydrolyzed by acid under conditions known to be necessary for the complete breakdown of the compound to hexose sugars. The reducing power of the neutralized hydrolysate was determined, and by expressing the reducing power of the digest as a percentage of the reducing power of the neutralized hydrolysate (making the

necessary corrections for dilutions and blank values), a measure of the takadiastase digestibility of these carbohydrates was obtained (table I). As will be seen, starch and maltose were almost completely hydrolyzed by takadiastase. In the case of Merck's yellow dextrin, however, only 52.7% was recovered as glucose by digestion with takadiastase. Further experiments with other commercial dextrins, all of which are produced by the action of acid upon starch, revealed that they were all more or less resistant to takadiastase (12). Dextrin produced from starch by saliva, on the other hand, was almost completely digestible by takadiastase. While this point needs further clarification, it may be assumed that the natural dextrins occurring in plants are likewise susceptible to takadiastase.

Inulin was found to be indigestible by takadiastase, and the new method of estimating total available carbohydrates is, therefore, not suitable for plant materials containing appreciable amounts of this carbohydrate. Inulin, which is only slightly soluble in water, will be retained in the residue and not included in the result. The partial breakdown of sucrose is doubtless due to the presence of invertase in takadiastase. As shown by previous workers, fructosans are likewise susceptible to invertase and also to takadiastase, though the action of invertase upon fructosans is very slow (2). It is, however, of no significance that the breakdown of sucrose and fructosans by takadiastase is incomplete, since these water-soluble carbohydrates go into solution during the process of digestion and are subsequently hydrolyzed by the treatment with acid.

EFFECT OF CLEARING AND HYDROLYSIS TREATMENTS ON SUGAR RECOVERY

In order to discover whether or not the recovery of sugars is affected by the clearing and hydrolysis treatments employed in the total available carbohydrate determination, the following series of tests was carried out: Aqueous solutions of glucose, fructose and sucrose were prepared containing approximately 40 mg. of the sugar per 100 ml. liquid volume. The latter included 35 ml. of buffer solution (pH 4.45); i.e., the same proportion of buffer solution as the digests. Solutions were subjected to the same clearing, deleading, and hydrolysis treatments as employed in the new method of determining total available carbohydrates. For the "clearing" treatment 250 mg. of powdered neutral lead acetate were used per 100 ml. solution.

In the case of fructose and glucose the reducing power of the solution was determined before treatment, after "clearing" and deleading, and again after "hydrolysis." The sucrose solution was divided into two portions, one of which was "cleared" and deleaded, while the other portion remained "uncleared."

The treatments resulted in no loss of sugar, and hydrolysis of sucrose was complete (table II). While the hydrolysis used here would appear somewhat more severe than the official methods of hydrolysis of sucrose, it should be mentioned that these experiments were carried out at an altitude

TABLE II

EFFECT OF CLEARING AND HYDROLYSIS TREATMENTS ON SUGAR RECOVERY
MILLIGRAMS SUGAR FOUND PER 100 ML. ORIGINAL SOLUTION

SUGAR	ORIGINAL SOLUTION	AFTER CLEARING AND DELEADING	AFTER HYDROLYSIS	AFTER FERMENTATION
	mg.	mg.	mg.	mg.
Glucose	43.0	43.6	43.4	0.00
Fructose	38.6	38.4	38.6	0.00
Sucrose				
"Uncleared"	41.9*	....	42.8	0.15
"Cleared"	41.9*	....	42.4	0.15

* Calculated value.

of 5,740 feet where the boiling point of water is not more than 93° to 94° C.
The temperature reading of the acidified sugar solutions during hydrolysis
was approximately 83° C. *At lower altitudes it may be necessary to modify
the conditions of hydrolysis slightly to avoid loss of fructose from sucrose
or fructosans.*

Aliquots of the neutralized hydrolysates were subjected to a fermenta-
tion procdure. The details of this procedure, as well as the results of the
fermentation experiments, are discussed in a subsequent section.

TESTS WITH PLANT MATERIALS

SUBSTRATE-ENZYME RATIO.—In the digestion of starch by takadiastase the
amount of enzyme used in relation to that of starch is an important factor.
DENNY (4) reported that complete conversion of starch to glucose was ob-

TABLE III

CARBOHYDRATE RECOVERY IN RELATION TO SUBSTRATE-ENZYME RATIO*

RHIZOMES OF Cynodon dactylon			MIXED GRASS ROOTS		
MATERIAL USED	GLUCOSE		MATERIAL USED	GLUCOSE	
mg.	mg.	%	mg.	mg.	%
50	8.6	17.2	200	11.2	5.60
75	13.5	18.0	300	17.2	5.73
100	17.8	17.8	400	22.7	5.68
125	22.1	17.7	500	28.2	5.63
150	26.7	17.8	600	33.7	5.62
200	33.8	16.9	700	39.7	5.67
300	49.8	16.6	800	45.6	5.70
400	64.4	16.1	900	51.4	5.70
....	....	....	1,000	55.8	5.58
WITHOUT TAKADIASTASE					
400	16.1	4.0	500	14.4	2.88

* Glucose values expressed as milligrams per digest and as percentages of the air-
dry material.

tained with a starch-takadiastase ratio of 1:2. Whenever plant materials of unknown carbohydrate content are to be analyzed, preliminary experiments should be carried out to ascertain the most suitable sample weight to be used.

Results of two typical series are discussed. The materials used were rhizomes of *Cynodon dactylon* and mixed roots of South African grasses. A series of digestions was carried out with each of the two materials, using amounts of air-dry material ranging from 50 to 1,000 mg. per digest. The total liquid volume was 30 ml. in all cases, including 10 ml. of dialyzed takadiastase solution, corresponding to 50 mg. original takadiastase. One flask was included in each series in which the takadiastase solution was replaced by 10 ml. of distilled water. This suspension was incubated and subsequently treated in exactly the same way as the proper digests. The glucose value of this extract hence represented the total amount of water-soluble, acid-hydrolyzable carbohydrates; *i.e.*, mainly sugars and fructosans.

In the case of Cynodon rhizomes satisfactory recovery was obtained with amounts of air-dry material ranging from 75 to 150 mg. per digest (table III). Percentage glucose values resulting from these digests agreed well within the limits of experimental error, and averaged 17.8% on the air-dry basis. Higher amounts of substrate gave low values; the relatively low value obtained with 50 mg. material per digest is probably due to an analytical error resulting from the use of such a small sample. The highest sample weight with which satisfactory carbohydrate recovery could be obtained was 150 mg. of air-dry material, and the total amount of glucose yielded in this digest was 26.7 mg. Hence, carbohydrate recovery was satisfactory if the ratio of total available carbohydrate to takadiastase was not appreciably smaller than 1:2. Since without the use of takadiastase only 4% of glucose was found, it is evident that $\frac{17.8 - 4.0}{17.8} \times 100 = 77.5\%$ of the total available carbohydrate was takadiastase-digestible; i.e., in the form of starch and dextrin.

The mixed grass roots were considerably lower in total available carbohydrate than were the Cynodon rhizomes and also contained less starch-dextrin in relation to total available carbohydrate, approximately 50% of the latter being extracted without the use of takadiastase. It is for these reasons that uniform results were yielded over the whole range of sample weights used; *i.e.*, up to 1,000 mg. of air-dry material. In this digest 55.8 mg. of glucose were found of which, however, only $(55.8 - 2 \times 14.4) = 27.0$ mg. were takadiastase-digestible carbohydrate, so that the actual substrate-enzyme ratio was still nearly 1:2.

For ordinary purposes it will usually be sufficient to conduct preliminary tests with two or three different sample weights. Even the result of a single determination can be accepted as reliable if the amount of total available carbohydrate found per digest is not appreciably greater than half the amount of takadiastase used.

COMPARISON OF METHODS

To test the reliability of the new method, 24 samples of shoots, roots, and rhizomes of grasses were analyzed by the new procedure as well as by one of the usual methods of carbohydrate fractionation. For the latter method sugars were extracted from the air-dry, killed material with 95% alcohol; fructosans and dextrins with cold water. For the determination of starch, saliva digestion and subsequent acid hydrolysis were used. Details of these methods and percentages of the individual carbohydrates of these materials have been published elsewhere (13). In order to compare the results with those of the total available carbohydrate determination by the new method, the combined percentages of sugars, fructosans, dextrins, and starch have been calculated as glucose, and are given together with an indication of the form of the principal carbohydrate(s) in each species (table IV).

TABLE IV

RESULTS OF CARBOHYDRATE DETERMINATIONS ON GRASSES

SPECIES	PRINCIPAL CARBO-HYDRATE(S)*	TOTAL AVAILABLE CARBOHYDRATE		UNFERMENT-ABLE RESIDUE
		FRACTION-ATION METHOD	TAKADIA-STASE METHOD	
SHOOTS				
		%	%	%
Brachiaria serrata	NRS	2.42	3.14	0.00
Hyparrhenia hirta	NRS	3.14	4.30	0.27
Themeda triandra	S	4.19	5.16	0.05
Trachypogon plumosus	NRS	2.50	4.00	0.57
ROOTS				
Agrostis tenuis	F	3.57	4.05	0.00
Arrhenatherum elatius	F	4.16	4.56	0.08
Brachiaria serrata	NRS	6.59	7.06	0.07
Cynodon dactylon	ST, NRS	3.99	4.76	0.00
Digitaria trichol.	NRS	7.37	8.26	0.07
Elyonurus argenteus	S	8.97	9.62	0.57
Eragrostis chalcantha	NRS	2.90	3.74	0.03
Harpechloa falx	NRS, ST	4.48	4.91	0.00
Lolium perenne	F	3.94	4.75	0.11
Microchloa caffra	NRS	2.07	2.77	0.07
Monocymbium ceres.	NRS	4.88	5.54	0.06
Pennisetum clandest.	S, F	14.08	16.10	0.00
Phalaris arundinacea	F, NRS	5.58	6.47	0.00
Trachypogon plumosus	NRS	4.00	4.95	0.09
Tristachya hispida	ST, NRS	10.66	11.29	0.09
RHIZOMES				
Agrostis tenuis	F	19.49	20.75	0.00
Cynodon dactylon	ST, NRS	18.12	19.80	0.09
Digitaria trichol.	NRS, ST	1.98	3.14	0.04
Pennisetum clandest.	S, ST	14.10	15.42	0.00
Phalaris arundinacea	F	36.89	38.85	0.00

* Abbreviations: F = fructosans; NRS = non-reducing sugars; S = sugars; ST = starch.

A comparison of the fractionation results with those of the takadiastase method shows that the latter produced higher results than the former in all materials. LOOMIS and SHULL (5) pointed out that takadiastase may hydrolyze materials other than carbohydrates and that high values may result from the production of reducing non-sugar substances. The proportion of these can be roughly estimated by fermentation, which will remove hexose sugars from such mixtures.

All the final extracts from the takadiastase digests (including blanks) were subjected to rapid fermentation by baker's yeast, essentially according to the procedure proposed by YEMM (14). A 20-ml. aliquot of the neutralized hydrolysate and 5 ml. of a 10% suspension of washed baker's yeast were mixed in a large test tube. The mixture was incubated for four hours at approximately 35° C., a steady stream of air being drawn through. The yeast was then separated from the solution by centrifuging. From the reducing power of the solution the unfermentable residue value of the extract was calculated, making the necessary corrections for blank values and dilutions. Preliminary fermentation tests with pure sugar solutions which had been subjected to the same clearing and hydrolysis treatments as the plant digests indicated that complete or almost complete removal of sugars could be attained by this procedure (table II). It is of particular interest to note that neither the use of thymol (introduced with the buffer solution) nor that of lead acetate for clearing inhibited the fermentation.

In most of the final extracts of the plant digests mere traces of unfermentable reducing substances were present (table IV). Only one instance was found where unfermentable reducing substances could possibly account for the higher carbohydrate value of the new procedure as compared with that of the orthodox method (roots of *Elyonurus argenteus*). In this material, however, the result obtained with the takadiastase method exceeded the fractionation result by only 7%. It would thus appear that the higher values yielded by the new method are, in general, due to fermentable sugars. The low figures for the unfermentable residues exclude particularly the possibility that any appreciable amounts of hemicelluloses were broken down by the takadiastase, since this would have resulted in the production of unfermentable pentoses. The high carbohydrate values yielded by the takadiastase method must, therefore, be due to the hydrolysis of carbohydrate groups such as occur in glucosides, proteins, pectic substances, and other colloidal materials. There seems to be no a priori reason why such carbohydrate groups, though not determined by the more commonly employed procedures, should not be included in the fraction of physiologically available carbohydrates in the plant body.

The correlation coefficient for the results of the fractionation and the takadiastase method, as computed from the 24 pairs of determinations, is + 0.9990, indicating a positive correlation of very high significance.

That differences in the total available carbohydrate content brought about by physiological conditions can also be ascertained by the new pro-

cedure is shown by the following series. Roots of *Tristachya hispida* which had been harvested from the plots of an experiment subjected to different clipping treatments for two seasons were analyzed (table V).

TABLE V

EFFECT OF DEFOLIATED INTENSITY ON CARBOHYDRATE CONTENT OF *Tristachya hispida* ROOTS

CUTS PER SEASON	COMBINED SUGARS-DEXTRIN-STARCH AS GLUCOSE*	TOTAL AVAILABLE CARBO-HYDRATE (TAKADIASTASE METHOD)
	%	%
1	12.97	13.93
2	11.15	12.60
4	8.60	8.37
9	1.57	1.49
16	0.95	0.97

* Sugars extracted with 80% alcohol; dextrin-starch determined on residue by saliva digestion and subsequent acid hydrolysis; fructosans absent in appreciable quantities.

Two series of experiments to assess the reproducibility of the new method were carried out. Ten determinations were done on a sample of mixed grass root material at intervals of several weeks, extending in all over half a year, thus necessitating the frequent use of separately prepared solutions of dialyzed takadiastase. The results are summarized, as follows:

Mean: 7.41% total available carbohydrate
Standard Error: ± 0.05% " " "
Standard Deviation: ± 0.14% " " "
Variability Coefficient: ± 1.89% " " "

For the second test duplicate determinations were carried out on 12 samples of roots and rhizomes of grasses, the total available carbohydrate content of which ranged from 2% to 20%. Results of nine of the twelve duplicate determinations agreed within 0.25% total available carbohydrate. The standard deviation was ± 0.15% total available carbohydrate, and the coefficient of variability ± 1.80% (calculated from the deviations of the single tests from their respective means). These two figures agree remarkably well with those of the first series. The data indicate that results of the new method can generally be reproduced to within 5%, which is sufficiently accurate for ordinary biological purposes.

Summary

A new method of estimating the total available carbohydrate content of plant material in a single determination is described.

Small samples of finely ground, air-dry material are digested by takadiastase under conditions resulting in the breakdown of starch, dextrins, and maltose to glucose, while other sugars and fructosans are extracted at the same time. After clarification of the digest the latter compounds are

converted to hexose sugars by acid hydrolysis, following which the reducing power of the neutralized hydrolysate is determined.

Results obtained with the new method were higher than the combined amounts of sugars, fructosans, dextrins, and starch, when determined separately and calculated as glucose. The reducing power of the digests was, however, almost completely due to fermentable sugars, the higher values obtained by the new procedure apparently resulting from the hydrolysis of carbohydrate groups not included in the more commonly employed methods of carbohydrate fractionation. Furthermore, the results of the total available carbohydrate determination showed a positive correlation of very high significance with those of the fractionation method.

The new procedure is simple and suitable for large-scale routine analysis; results can, in general, be reproduced to well within 5%.

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LITERATURE CITED

1. ALDOUS, A. E. Relation of organic food reserves to the growth of some Kansas pasture plants. Jour. Amer. Soc. Agron. **22**: 385–392. 1930.

2. ARCHBOLD, H. K. Fructosans in the monocotyledons. A review. New Phytologist **39**: 185–219. 1940.

3. BUNTING, A. H. A necessary precaution in the use of takadiastase for the estimation of maltose. Biochem. Jour. **36**: 639–640. 1942.

4. DENNY, F. E. Improvements in methods of determining starch in plant tissues. Contrib. Boyce Thompson Inst. **6**: 129–146. 1934.

5. LOOMIS, W. E., and SHULL, C. A. Methods in plant physiology. Mc-Graw-Hill Book Co., Inc. New York and London. 1937.

6. MCCARTY, E. C. Seasonal march of carbohydrates in *Elymus ambiguus* and *Muhlenbergia gracilis*, and their reaction under moderate grazing use. Plant Physiol. **10**: 727–738. 1935.

7. —————. The relation of growth to the varying carbohydrate content in mountain brome. Tech. Bull. **598**. U. S. Dept. of Agr., Washington, D. C. 1938.

8. —————, and PRICE, R. Growth and carbohydrate content of important mountain forage plants in Central Utah as affected by clipping and grazing. Tech. Bull. **818**. U. S. Dept. of Agr., Washington, D. C. 1942.

9. WEINMANN, H. Effects of defoliation intensity and fertilizer treatment on Transvaal Highveld. Emp. Jour. Exp. Agr. **11**: 113–124. 1943.

10. —————. Semimicro estimation of reducing sugars. Plant Physiol. **19**: 148–156. 1944.

11. —————. Root reserves of South African Highveld grasses in relation to fertilizing and frequency of clipping. Jour. South African Bot. **10**: 37–54. 1944.

12. —————. Behavior of dextrins towards takadiastase. Jour. Biol. Chem. **164**: 7–10. 1946.

13. —————, and REINHOLD, L. Reserve carbohydrates in South African grasses. Jour. South African Bot. **12**: 57–73. 1946.

14. YEMM, E. W. The respiration of barley plants. I. Methods for the determination of carbohydrates in leaves. Proc. Roy. Soc. London, Series B, **117**: 483–504. 1935.