STUDIES ON THE CARBOHYDRATE METABOLISM OF GRASSES IN RELATION TO NITROGEN METABOLISM.

G.I. AMOS.

Thesis for the Degree of Master of Science. -

Adelaide University.

(Presented on the 1st of September, 1938).

My husband died in 1967.
I gladly give my consent to photo copying.
It is possible that my son at Melbourne Uni
may wish to refer to it as he is working on
husbands the same, since he has
PHD

I hereby consent that my Thesis

(title): STUDIES ON THE CARBOHYDRATE METABOLISM OF GRASSES IN RELATION TO NITROGEN METABOLISM.

.....

.....

.....

be made available for loan and/or photo-copying.

Signature:

Date: 7/1/77

TABLE OF CONTENTS.

INTRODUCTION.

storical.

Prior to 1936, no complete systematic examination of the relationships between the carbohydrate fractions of grasses had been undertaken, nor had they been considered in relation to the various nitrogen fractions.

Kerr (1) reported that addition of ammonium sulphate as fertilizer to a field crop led to a decrease in the sucrose content of sugar cane (Saccharum officinarum). Orcutt and Wilson (2), working with soy beans, reported that a medium nitrogen supply results in an increase of soluble sugars, whereas a high or low nitrogen level reduces the soluble sugar content.

Gregory and Baptiste (3) first studied the effect of nutrient deficiency on the hexose and sucrose concentrations in barley. They grew their plants in pot cultures and subsequently estimated sucrose and hexose by the classical method of Hagedorn and Jensen. They concluded that nitrogen deficiency had no consistent effect on free reducing sugar, but increased total sugar. Their results, however, for two reasons leave much to be desired. Firstly, the sucrose and total reducing sugar concentrations do not provide sufficient evidence on which to base a theory of the course of carbohydrate metabolism in the plant, and secondly, the non-sugar reducing substances, which may amount to as much as 30% of the total reducing substances as estimated by the Hagedorn Jensen method, have not been taken into account.

In a further study, Gregory and Sen (4) concluded that, in the leaves of barley, water content was reduced, respiration rate was greatly reduced, and increase in sugar concentration occured under conditions of nitrogen deficiency. A scheme was proposed for the interrelationships between the various carbohydrate and nitrogen fractions, but the objections outlined above apply to their analyses as to their previous results(3).

Archbold (5), realizing that earlier carbohydrate analyses were neither

accurate nor complete enough for an increased understanding of [illegible] metabolism in plants, re-examined the methods of estimation of the various fractions which had been previously employed. She also realized the necessity for constructing a complete carbohydrate balance sheet, and the importance of the water - soluble, labile fructosan, previously examined by her in 1935 (6). In a further contribution, Archbold (7) estimated sucrose, reducing sugar and fructosan, over the complete growing period ofbarley, and noted the seasonal variation in these substances.

The relationships between the various nitrogen fractions of grasses have been considered by Petrie and Wood in a series of contributions (8, 9, 10,), and their experimental method has been adopted in this research. The aim of the experiment was to construct as complete a carbohydrate balance sheet as possible and to consider its relationship to the two nitrogen fractions, amino and protein nitrogen. The following fractions were estimated: glucose fruchose, sucrose, fructosan, hemicellulose, starch, water, acidity of expressed sap, respiration rate, protein - nitrogen and amino - nitrogen, thus, compiling a more comprehensive record than has hitherto been attempted in the one set of plants.

2. Experimental Method.

Plants were submitted to an environment as near constant as possible with regard to temperature, light, and water supply, and certain factors, the effect of which it was desired to investigate, were varied. As in the experiments of Petrie and Wood (8, 9, and 10), conditions were such that a steady state was approached in the leaves much more closely than had the plants been subjected to a fluctuating environment. Also, as these investigators pointed out, a constant steady state is not to be expected in this type of experiment, the drift in the composition of the external nutrient solution alone being sufficient to prevent it. However, it was hoped that an approach to a drifting, steady

state, where the effect of the varying factors is great in comparison with the drift in the factors which determine the steady state, would be attained.

At daily intervals, a certain number of plants were examined to determine the various carbohydrate and nitrogen fractions, water content, respiration rate, and pH of the expressed sap.

MATERIALS.

ation of Material.

Seeds of a pure line of Wimmera Rye Grass (Lolium subulatum) were planted out in seed boxes containing washed sand. After germination , and when the cotyledon was about 1½ inches high, the seedlings were transferred to glass pots. Each pot contained 3.5 kilos. of sand. Two samples of the sand were taken, and the amount of water required for saturation determined.

Eight even seedlings were chosen from the seedboxes and transplanted in each pot. Sufficient distilled water was added to maintain 70% saturation of the sand. 180 such pots were prepared. When the plants had become established, the pots were thinned out to four even plants per pot, and the surface was covered with loose gravel. 70% saturation was maintained by weighing the pots twice per week, and bringing up to a previously determined constant weight with distilled water. Each pot was covered with opaque paper blackened on one side, so that only the upper surface was exposed to the light. The pots were placed in a glasshouse and were evenly spaced to avoid shade and lighting effects. The following applications of nutrient solutions were added at the times indicated: First application (added at the end of the first week after transplanting).

$Ca(H_2PO_4)_2$	.3 grms. per pot.
K Cl	.15 " " "
$(NH_4)_2 SO_4$	.3 " " "
$Mg SO_4 . 7H_2O$	.15 " " "
$Fe Cl_3$	.015 " " "
$Mn SO_4 . 4H_2O$	.0003" " "

Second Application (about a fortnight later).

$Ca(H_2PO_4)_2$	.3 " " "
K Cl	.15 " " "
$(NH_4)_2 SO_4$	.3 " " "
$Mg SO_4 . 7H_2O$	.15 " " "

Third application (three weeks later).

$(NH_4)_2 SO_4$	.3 " " "
Na OH	.06 " " "

Fourth application (two weeks later)

$(NH_4)_2 SO_4$	.15 " " "
NaOH	.06 " " "

The use of caustic soda in the third and fourth applications was to neutralize acids formed due to the rapid uptake of the ammonium ion.

Stock solutions were made up in Winchesters, and appropriate aliquots used.

A. 1 litre containing 15 grms. $Ca(H_2PO_4)_2$ per litre.

20 ml. diluted to 200 ml. for each pot.

B. 1 litre containing 15 grms. KCl

30 " $(NH_4)_2 SO_4$

15 " $Mg SO_4 . 7H_2O$.

10 ml. diluted to 100 ml. for each pot.

C. 1 litre containing 1.5 grms. $FeCl_3$

.03 " $MnSO_4.4H_2O$

10 ml. diluted to 100 ml for each pot.

Two large cabinets were prepared for holding the pots over the period of differential treatment. Each consisted of a double jacketed wooden cabinet painted white inside and with a clear glass roof. Four powerful electric light globes with reflectors attached were suspended above the glass roof, and the heat generated was absorbed by a constant stream of cold water flowing over the plate glass. The air in the cabinet was circulated continuously by an electric fan. Although most of the heat from the lamps was absorbed by the water stream, sufficient passes through to act as a source of heat, which, in combination with an automatic refrigerating system enabled the temperature to be maintained constantly at $(24 \pm .5)^\circ$ C. The refrigerator was automatically brought into operation by means of a bimetallic strip make and break in the refrigerator circuit, and the circulating air passed over it and the circulating air passed over it until the temperature had fallen to approximately 23.8°c. To prevent the humidity from rising excessively and to ensure a sufficient supply of carbon dioxide, the nozzle of a blower was introduced into each cabinet, thus maintaining a constant supply of new air. A shelf was arranged in each cabinet to provide two light intensity levels, the pots receiving lower light intensity resting on the floor of the cabinet. After allowing for the height of the pots, the intensity at each level was determined, and although not constant at either level, the lowest value at the higher light level was greater than the highest value at the lower level. Continual observation was facilitated by double doors, the inner ones being of double sheets of glass.

A preliminary run showed that the temperature could be maintained constantly at 24° C. with a variation not greater than $\pm \frac{1}{2}$°c. There was no temperature gradient between the two light levels. Cabinets were allowed to run for a full day under experimental conditions before the plants were placed in them.

3. Additional treatment of experimental plants.

Pots which contained four even plants were chosen for experimental treatment. The treatment to be received was written on wooden labels, which were randomized, and allotted to the pots.

The treatments were as follows.

Day 1.

L_1N_o L_1N_1 L_1N_2

L_2N_o L_2N_1 L_2N_2

Day 2. Same as for day 1.

Day 3. Same as for day 1.

Day 4.

L_1N_1 L_1N_2

L_2N_1

For each treatment 4 pots, each containing 4 plants were used

L_1 = high light intensity (.???....metre candles)

L_2 = low light intensity (..???....metre candles)

N_o = no additional nitrogen.

N_1 = 2 grms. additional $(NH_4)_2 SO_4$ per pot.

N_2 = 6 " " " " "

All pots were transferred from the glasshouse, and two of each treatment were placed in either cabinet to eliminate errors due to slight differences in the cabinets. Prior to placing the pots in the cabinets they were adjusted to 70% saturation.

Next morning the additional treatment was added to the appropriate pots, the N_1 series receiving two, and the N_2 series six grams of ammonium sulphate in solution.

Harvest Procedure.

The first harvest was conducted during the following day. The four pots for each treatment were taken separately from the cabinet, one plant from each cabinet being used for respiration rate determinations, the others for analysis. Each plant was cut off at the base, and all from one pot were transferred to a tin containing a layer of damp absorbent paper. The tins were rapidly transferred to the laboratory where the leaves were cut off at the ligule, and all from one pot were weighed separately. All were then bulked, chaffed and mixed. Two lots of 10 grms. each were then added to 75 ml. boiling 95% alcohol in a 250 ml., Erlenmeyer flask to destroy enzymes. A pinch of calcium carbonate was added to neutralize plant acids. The samples were added a little at a time so that the boiling was not interrupted. The boiling was allowed to continue for 20 minutes under reflux, the flasks then being removed and labelled clearly for subsequent carbohydrate analysis.

Five to ten grams of the chaffed material were taken to a Buchner press, the sap was extracted, and the pH was determined by means of a Cambridge glass-electrode pH meter.

About 25 grms, were dried in an air oven for the determination of the dry weight.

At the conclusion of ~~the~~ each harvest, which was repeated on four consecutive days, the remaining pots in the cabinets were brought up to 70% saturation, and blanks were substituted for the pots used during the day.

ANALYTICAL TECHNIQUE.

.. Comparison of Sugar Methods.

There are two methods in general use for the determination of reducing sugars, one depending on the reduction of the cupric to the cuprous ion (11, 12, 15) and the other being concerned with the oxidation of the reducing group with potassium ferricyanide in alkaline solution (13). If, however, both of these methods are applied to the same plant extract, higher figures are obtained using the alkaline ferricyanide method than with the copper reduction. This was found to be due to the presence of non-sugar reducing substances, since on fermentation of the cleared plant extract with washed yeast, and subsequently determining the reducing capacity of the solution, it is found that up to 30% of the reducing capacity of the original extract, as determined by the alkaline ferricyanide method, is due to these unidentified substances. The copper method is not so sensitive. When yeast fermentations are performed on the grass leaf extracts, and the final reduction of the solution is substracted from the original, both methods give almost identical results. (Table 1.)

TABLE 1.

Total reducing sugar in rye-grass cut from the field.

METHOD OF BENEDICT.			MODIFIED HAGEDORN-JENSEN METHOD.		
% reducing substances glucose equiv.	non-sugar reducing substances glucose equiv.	% reducing sugar	% reducing substances glucose equiv.	non-sugar reducing substances glucose equiv.	% reducing sugar.
4.24	.37	3.87	5.70	1.85	3.85
4.82	.53	4.29	6.31	2.07	4.24
4.49	.50	3.99	6.07	2.03	4.04

In table 1, a comparison is given between the reducing sugar values of Lolium subulatum as determined by both methods. Samples were taken from the field on 3 different days and extracted as described. Reducing capacity before and after fermentation with washed baker's yeast was estimated by the method of Benedict (11) and a modified Hagedorn Jensen method. The alkaline ferricyanide reagent is much more sensitive toward the non-sugar reducing substances than is the copper reagent, thus accounting for the higher figures using the former. However, substraction of the glucose equivalent of the residual reducing power after fermentation from that of the original solution shows that either method gives reliable results for the actual reducing sugar.

In this study, the alkaline ferricyanide method was chosen because of its ready adaptability to the whole carbohydrate series. The principle involves hydrolysing the carbohydrate fraction to some reducing form and estimating the reducing capacity of the resultant solution.

REAGENTS.

a. 10% zinc sulphate $ZnSO_4 . 7H_2O$

b. .5 N Caustic soda

c. .2 N sulphuric acid

d. .01% aqueous soln. phenol red.

e. 1N caustic soda.

f. 3% acetic acid.

g. Alkaline ferricyanide reagent

Soln. A. 3.30 grams pot. ferricyanide purified with brominated alcohol and ether according to Peters and Van Slyke (16), dissolved in 1 litre of water and stored in a dark bottle.

Soln. B. 21.20 grms. anhydrous sodium carbonate dissolved in 1 litre of water.

Mix equal volumes A and B before use.

h. Iodide - zinc reagent.

Dissolve 250 grms. sodium chloride and 50 grms. $ZnSO_4 . 7H_2O$ and make up to 1 litre. Before using add 2.5 grms. solid potassium iodide to 100ml. of this solution.

i. Sodium thiosulphate solution.

Dissolve 24.82 grms. sod. thiosulphate in CO_2- free water and make up to 1 litre. This gives a .1N solution. Store as such and dilute 10ml. to 200 ml. before use. The diluted solution does not keep.

j. Starch indicator solution.

1% starch solution in saturated N a Cl.

k. Potassium iodate solution .005N.

For standardizing thiosulphate soln. take 5 ml. Add 3 ml. iodide-zinc reagent and 2 ml. 3% acetic acid. Titrate thiosulphate. The correction factor to be applied to estimations is

$$\frac{5}{\text{no. of ml. } Na_2S_2O_3}$$

l. Glucose oxidation reagent.

Dissolve 16.7 grms. potassium iodide and 8.47 grms. iodine and make up to 1 litre.

m. .167 N caustic soda.

n. .02 - .04 N. sodium sulphite.

Make up to .5N solution in small quantities. Store in a tightly corked bottle and dilute as required.

o. .25N. sulphuric acid.

p. Solution of β- amylase.

Grind ungerminated barley of good malting quality to a fine flour in a mill. Throughout this preparation use

metal free distilled water from an all Pyrex still. Extract 200 grms. flour with 750 ml. 50% alcohol by stirring for 2 hours. Centrifuge. Pour off the supernatent fluid into a vessel and re-extract the residue with 400 ml. 50% alcohol for half an hour. Centrifuge. Combine the supernatent liquors, Filter, and increase the concentration of alcohol to 80%. Centrifuge. Discard supernatent liquor and draw the precipitate on a suction filter. Divide finely and suspend in 450 ml. water. Add a few drops of toluene and store in the dark at 1°C. After 3 days, filter, The solution retains its activity for months.

q. Acetate buffer of pH 4.63 (22)

r. 2% sulphuric acid.

ination of Reducing Sugars and Sucrose.

A. Total reducing capacity.

From the samples treated for carbohydrate analysis, filter off the alcohol and re-extract the residue 3 times with 75 ml. 90% alcohol, each extraction occupying 2½ hours. Continue extracting until all the chlorophyll has disappeared from the residue. Combine the extracts in a round bottomed distillation flask fitted with a splash-head by a ground glass fitting. Dry the residue in an air oven and set aside for further analysis. From this point onwards, 5 estimations and 1 blank may be carried through simultaneously. From the combined extracts distil off the alcohol under reduced pressure at approx. 40°C. The remaining material is taken up in water and made up to 100 ml. 10 ml. are transferred to a 100ml. standard flask. Add 1 ml. 10% zinc sulphate and 1 ml. .5N caustic soda. This was found to effect a much better clearing of these solutions than basic lead acetate.

The volume is made up to 100 ml. and filtered. Estimations of total reducing sugars, non-sugar reducing substances, fructose, glucose and sucrose may be made on this filtrate. Do each in duplicate.

For total reducing capacity, take 5 ml. of the filtrate; for reducing capacity after inversion dilute 10 ml of the filtrate to 100 ml. and take 10 ml. of the resultant solution. Pipette these aliquots into large test tubes graduated at 15 ml. To the solutions to be inverted, add 2 ml. .2N sulphuric acid. Put tubes in a wire basket and immerse in a boiling water bath for 10 minutes. Inversion was found to be completed in this time. Cool tubes and add 1 drop of .01% phenol red. Add 1N caustic soda solution drop by drop until solution turns red and bring back to pale yellow colour with .2N sulphuric acid also added drop by drop.

Add 5 ml. alkaline ferricyanide reagent to all tubes for determination of reducing substances both before and after inversion. Make up to the 15 ml. mark. Heat on a boiling water bath for 15 minutes and allow to cool. In this time, all the reducing sugar is oxidized and no change occurs in the cooled solutions even after standing for 4 hours. Add 3 ml. iodide-zinc reagent, 2 ml. 3% acetic acid and set aside for 2 minutes. Titrate approximately .005 N thiosulphate solution from a microburette until the yellow colour of the liberated iodine has almost disappeared. Add 1-2 drops of starch indicator solution and continue the addition of thiosulphate solution until the reddish-purple colour just disappears. Standardize the thiosulphate solution against standard .005 N potassium iodate. Subtract the titration figures obtained from the blank and apply the correction factor to obtain the number of ml. of .005 N thiosulphate equivalent to the amount of reducing sugar present. Walkley (17) reinvestigated the table constructed by Hagedorn and Jensen, and over the range of sugar

concentrations used in these analyses, the ratio

$$\frac{\text{mg. glucose.}}{\text{no. of ml. thiocyanate}} = K = .1814$$

This conversion factor has been used throughout for glucose equivalents. Hence the glucose equivalent before and after inversion is given by

(B - R) x correction factor x .1814 x dilution.

where B = blank reading and R = titration figure.

B. Fructose

The determination of fructose before and after inversion was carried out in case any of the labile fructosan had been extracted in the alcohol extract. The method is based on the preferential oxidation of glucose by iodine as discussed by van der Planck (18) and subsequent estimation of the reducing sugar in the solution by oxidation with alkaline ferricyanide.

10 ml. of the cleared filtrate was measured into a 25 ml. standard flask and 10 ml. of the diluted filtrate was hydrolysed and neutralized as before. All flasks were transferred to the refrigerator, where all reagents involved in the oxidation were stored. When cooled to about 2°C, 2 ml. of the glucose oxidizing reagent was added. The contents of the flask were rotated and 1 ml. .167 N caustic soda was added drop by drop and the neck of the flask was washed down with 1 ml. water. The stopper was replaced and the contents allowed to remain at 1° - 2°C for 2 hours. This was followed by acidification with 1 ml. .25 N sulphuric acid, and excess iodine was removed with .02 - .04 N sodium sulphite using 1 drop of starch indicator solution. After neutralization with 1 ml. .23 N caustic soda, the volume was made up to 25 ml. with distilled water.

10 ml. aliquots were then treated as before, and total fructose before and after inversion were calculated making allowances for the corrections mentioned by van der Planck (18)

C. Non-sugar reducing substances.

The estimation of non-sugar reducing substances is based on the removal of fermentable sugars with yeast. 40 grms. of moist baker's yeast cake are suspended in water and centrifuged. The supernatent liquid is poured off and the washings repeated until the supernatent fluid is clear. 4-5 washings are usually sufficient. The residue is suspended in 400 ml. distilled water and kept in the refrigerator.

Hydrolysis was found to increase the reducing capacity of the non-sugar reducing substances and hence, after yeast fermentation, estimations of reducing capacity must be done on both hydrolysed and unhydrolysed aliquots.

Centrifuge 10ml. yeast suspension and pour off the supernatent liquid. Dry the sides of the centrifuge tubes with strips of filter paper. Add 25 ml. plant extract and mix up well. Place in a water bath at 37° - 39° C for 20 minutes. Kepp well stirred. Centrifuge off the yeast and take aliquots of the supernatent fluid for the estimation of reducing capacity both before and after hydrolysis with .2 N sulphuric acid as previously outlined. Calculate glucose equivalents and subtract from corresponding figures in previous determination. Express glucose, fructose and sucrose as percentages of the dry weight.

Determination of Polysaccharides.

A. Fructosan.

It has long been known that fructose anhydrides occur in cereals (6), its isolation from the leaves of barley was performed by Archbold and Barker (6) and it was discussed by Yemm (20). It is soluble in cold water and insoluble in 80% alcohol. The following method was constructed for its estimation.

Place the residue from the alcoholic extraction in a watertight stoppered bottle with 80 ml. water and shake in a mechanical shaker for 4 hours.

Filter, wash, and make filtrate up to 200 ml. in a standard flask. Take an aliquot of 10 ml., hydrolyse with .2N sulphuric acid, and determine glucose and fructose separately. This showed that the fructosan regularly contained approx. 6% glucose, so after a short time the two estimates were discontinued, and the total reducing capacity figures were used to estimate the fructosan. Non-sugar reducing substances in the cold water extract were also estimated by fermentation with yeast as before. The difference represents hexose from fructosan hydrolysis.

B. Starch.

The residue from the fructosan analysis is transferred to a flask, boiled with 60 ml. distilled water and allowed to cool. The following method is based on an observation by Hanes (21) that 60% of the starch molecule is hydrolysed to maltose by the saccharogenic component of malt diastase, β - amylase.

To the flasks containing the suspension of plant material, add 10 ml. acetate buffer (22) to maintain digests at optimal pH of 4.63. Add 10ml. β - Amylase solution and a few drops of toluene to prevent bacterial infection. Plug flasks with cotton wool, flame, and allow to stand at 25°C for 48 hours.

No preliminary solubilisation of the starch is necessary, because the extraction of the fresh leaf material with 95% alcohol and the boiling prior to this estimation accomplishes it. All pipettes used were plugged with cotton wool to exclude traces of saliva. Hanes suggests that increase in reducing power should be determined by the difference in reduction values of parallel digests with active and "killed" enzyme, the latter being inactivated by holding at 90°C. for 10 minutes. Such inactivation, however, undoubtedly alters the structure of the enzyme molecule, and almost certainly its reducing capacity. This alteration takes place under different conditions to those existing when the active

enzyme is killed, i.e. during heating in the presence of the alkaline ferricyanide reagent. Hence the method adopted in this research was to determine the difference in reducing capacity between parallel digests of plant material and distilled water, in which case both lots of enzyme were inactivated at the same time and had the same reducing effect on the alkaline ferricyanide reagent.

After 48hours remove the digests from the constant temperature apparatus, filter, clear and filter again. Make up to 200 ml. and take a 10ml. aliquot of the filtrate for the determination of maltose. Use the modified Hagedorn Jensen method described above, and use the relationships that 1c.c. .005 N thiosulphate is equivalent to.212 mg. anhydrous maltose. Since this represents only 60% of the starch molecule, multiply by a correction factor of $\frac{5}{3}$ to convert maltose to starch.

C. Hemicelluloses.

In this study, the heterogeneous hemicelluloses have been estimated as the glucose equivalent of the reducing capacity of the individual units. Assuming that the relative number of reducing to other units is constant, the figures obtained are useful for comparative purposes, but they are not claimed to be accurate measures of the absolute quantities of hemicelluloses present.

The residue from the starch digestion is returned to the flask and extracted by boiling under reflux with 100 ml. 2% sulphuric acid for 6 hours. This hydrolyses hemicellulose to its constituent units. Filter. Make filtrate up to 200 ml. Take 10 ml. and dilute to 100 ml. Take 10 ml. of the diluted filtrate, neutralize to phenol red as before, and determine reducing capacity. Calculate the glucose equivalent of the reducing capacity and express as a percentage of the dry weight.

D. Crude fibre.

The residue from the hemicellulose digestion is well washed and allowed to stand in 500 ml. distilled water overnight to remove traces of sulphuric acid. Dry in an air oven and weigh.

The above scheme for carbohydrate analysis of fresh plant material provides a more comprehensive scheme than has hitherto been presented. It is convenient in that, after treatment, all fractions are finally estimated by the one method - the reducing action as measured by the amount of ferricyanide reduced. The effect of all added reagents is accurately checked by using a distilled water blank throughout.

ANALYTICAL RESULTS.

The analytical figures are presented in Table 2. If the high light intensity is represented as L_1, the low light intensity as L_2, and the nitrogen treatments as N_o (no additional nitrogen treatment), N_1 (2 grms. additional ammonium sulphate per pot), N_2 (6 " " " " " "), then the treatments are as follows:-

Treatment	A	L_1N_o
"	B	L_1N_1
"	C	L_1N_2
"	D	L_2N_o
"	E	L_2N_1
"	F	L_2N_2

TABLE 2.

ANALYTICAL RESULTS.

Preliminary.

Water	521
Protein - N	2.67
Total amino - N	n.d.
Sucrose	11.89
Fructosan	3.56
Hemicellulose	11.53
Starch	0.84
Glucose	1.59
Fructose	nil
Crude fibre	n.d.
Respiration rate	n.d.
pH	6.42

	Treatment.					
Day 1	A.	B.	C.	D.	E.	F.
Water	610	627	512	643	652	673
Protein - N	2.39	2.77	2.49	2.50	2.68	2.74
Total amino - N	0.103	0.220	0.492	0.097	0.183	0.219
Sucrose	9.69	6.44	7.39	4.89	3.83	3.69
Fructosan	2.46	1.25	3.62	3.02	1.08	0.97
Hemicellulose	13.29	12.85	11.81	13.18	12.93	12.83
Starch	0.75	0.60	0.33	0.59	0.61	0.51
Glucose	1.55	1.13	1.65	0.90	1.11	1.09
Fructose	1.26	1.78	1.69	2.54	2.46	1.68
Crude fibre	0.373	0.369	0.432	0.371	0.375	0.372
Respiration rate	2.00	1.76	3.03	2.20	2.10	2.90
pH	5.72	5.65	5.48	5.72	5.63	5.48
Day 2						
Water	637	627	538	690	676	637
Protein - N	2.46	2.87	2.69	2.53	2.69	2.67
Total Amino - N	0.093	0.164	0.271	0.130	0.141	0.181
Sucrose	6.13	5.30	5.60	3.85	3.04	3.85
Fructosan	1.81	1.30	0.96	0.70	0.52	1.34
Hemicellulose	13.45	12.14	10.86	13.12	12.65	11.95
Starch	0.59	0.93	0.62	0.68	0.56	0.38
Glucose	1.30	1.35	1.55	1.05	0.82	0.26
Fructose	1.25	1.59	1.42	1.58	0.71	2.34
Crude fibre	0.392	0.384	0.442	0.381	0.366	0.363
Respiration rate	2.66	2.41	3.34	3.40	2.32	3.25
pH	5.62	5.73	5.59	5.71	5.75	5.52

TABLE 2 (Contd.)

	Treatment.					
Day 3.	A	B	C	D	E	F
Water	614	636	505	719	652	583
Protein - N	2.21	3.08	2.67	2.40	2.82	2.77
Total amino - N	0.096	0.168	0.272	0.114	0.228	0.380
Sucrose	7.08	4.54	5.33	2.44	3.67	2.78
Fructosan	2.12	0.26	1.00	0.35	0.72	0.26
Hemicellulose	13.58	12.32	11.60	13.12	12.64	11.48
Starch	0.59	0.64	0.52	0.43	0.57	0.42
Glucose	1.35	0.93	1.03	1.15	1.25	0.66
Fructose	1.32	1.51	1.82	1.17	2.09	1.18
Crude Fibre	0.387	0.389	0.422	0.379	0.362	0.360
Respiration rate	1.94	1.60	2.14	1.75	1.21	1.75
pH	5.63	5.72	5.55	5.62	5.70	5.52
Day 4.						
Water	-	603	546	-	712	-
Protein - N	-	2.93	3.01	-	2.86	-
Total amino - N	-	0.181	0.302	-	0.181	-
Sucrose	-	5.37	4.80	-	2.91	-
Fructosan	-	0.92	0.39	-	0.40	-
Hemicellulose	-	12.29	11.12	-	11.01	-
Starch	-	0.60	0.54	-	0.54	-
Glucose	-	1.55	1.66	-	0.66	-
Fructose	-	1.09	0.80	-	1.53	-
Crude fibre	-	0.395	0.413	-	0.368	-
Respiration rate	-	2.20	2.61	-	1.76	-
pH	-	5.68	5.60	-	5.68	-

Water, Carbohydrate and nitrogen fractions in per cent dry weight.
Respiration rate in mgs. CO_2 per gm. per hour.
Crude fibre in gms. per 10 gm. fresh material.

PRESENTATION OF THE DATA.

A. Drifts with Time.

The drifts in the fractions with time are illustrated in fig. 1, and that of the reserved carbohydrates in fig. 2.

TIME DRIFT OF VARIOUS FRACTIONS.

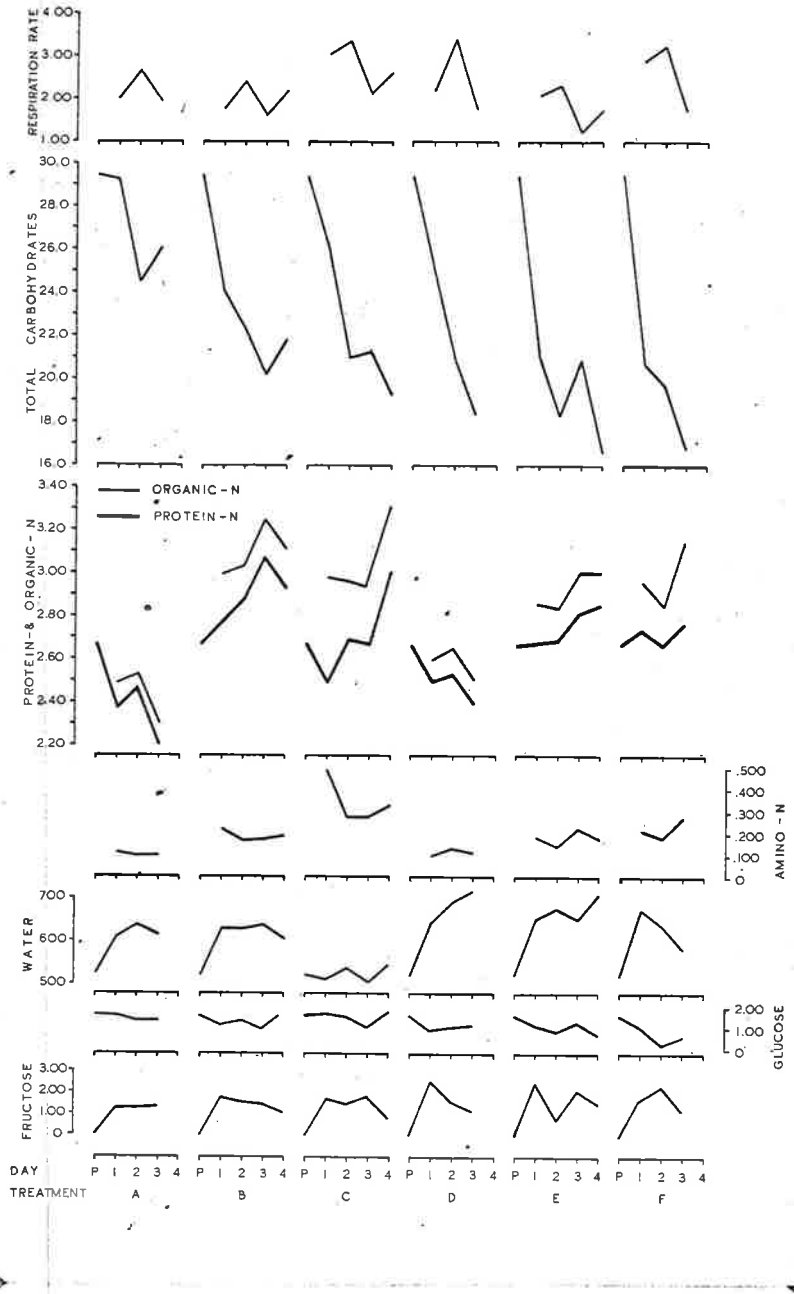


Fig. 1. Abscissae P - 4 represent one-day intervals. Ordinates represent % dry weight except respiration (mg. CO_2 per gram per hour.)

The preliminary respiration rate was not measured; all other figures are in mg. CO_2 per gram per hour. In all treatments the respiration rate rises on the second day, reaching its highest level in treatments C, D, and F. On the third day it falls again, and in all cases rises on the fourth day.

The total carbohydrates do not begin to fall off quite so rapidly under high light intensity (Treatments A, B, and C.) as under low (Treatments D, E and F), and the fall is more rapid in the presence of added nitrogen than without. Treatment A, which received no additional nitrogen remains the same throughout the first day and then begins to fall but not to such a low level as do the other treatments. Treatment B continues falling until the third and then rises slightly. Treatment C continues falling. Treatments D, E and F all fall very rapidly on the first day, D and F, in which the respiration rate is high, continue falling, and E in which the respiration rate reaches its lowest values, does not fall as regularly after the initial depletion.

Protein nitrogen and total organic nitrogen reflect the effect of treatment in their drifts. Under conditions of no additional nitrogen a continual depletion occurs. Under high light intensity and added nitrogen an evident synthesis of protein has taken place and under low light intensity, where the energy available for synthesis is least, and the carbohydrate reserves are most severely depleted, the levels have not been so markedly increased with time but they show a tendency to do so.

The preliminary concentration of amino acids was not measured, but they have rapidly adjusted themselves to the new conditions, and thereafter remained relatively constant. Treatments A, B, and C show increasingly higher levels, as do treatments D, E and F.

The water content shows a marked initial rise in all cases except in treatment C. Under high light intensity, equilibrium has been rapidly

reached, but in the final three treatments the water contents rose to a higher level than in the corresponding high light treatments. Again high nitrogen treatment shows a decline in the watef content.

The glucose figures remain fairly constant under treatments A, B and C with a slight tendency to fall off with time. Under low light the fall is more pronounced, particularly where the nitrogen figures are high and total carbohydrate is severely depleted. Fructose in all cases shows an initial rise, and then remains fairly steady with a tendency to fall off with time.

B. Time drifts of the Reserve Carbohydrates.

The drifts with time of starch, hemicellulose, sucrose and fructosan are illustrated in Fig 2. The general depletion of total carbohydrate is thus seen to be mainly due to depletion of the water soluble, labile, reserves, sucrose and fructosan.

TIME DRIFTS OF RESERVE CARBOHYDRATES.

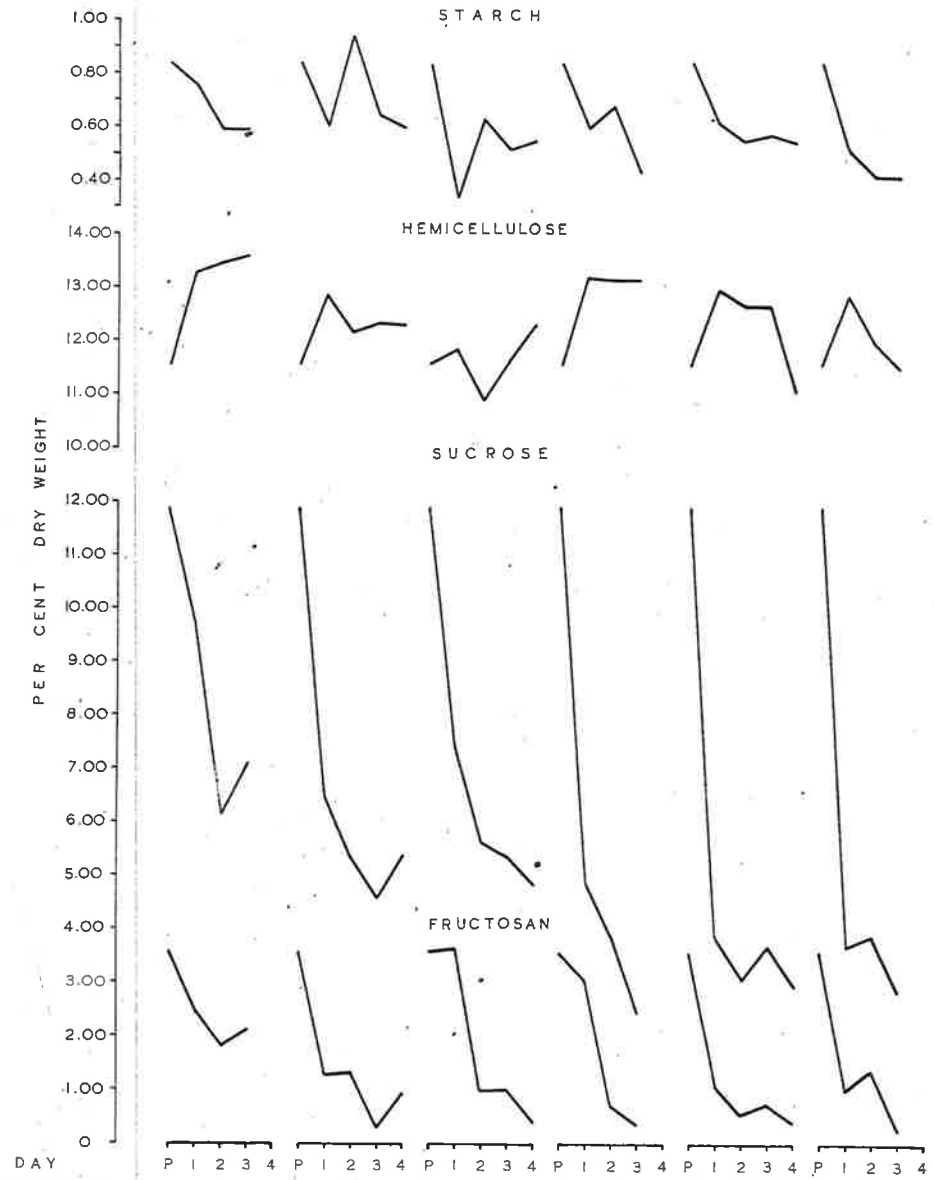


Fig. 2. Abscissae P - 4 represent one-day intervals.

Ordinates represent percentage of dry weight.

The starch figures are rather irregular, but in general they show a falling off with time, more regularly under low than under high light intensity.

Where no extra nitrogen has been added, the hemicellulose shows an initial increase and then remains steady, whereas when additional nitrogen has been added, the hemicellulose falls off to lower values after the initial rise. This sharp initial rise in the case of hemicellulose is paralleled by the equally sharp initial rise in the water content. Under treatment A, both hemicellulose and water undergo a slight rise over the next two days. With treatment B, the water content remains fairly steady whereas the hemicellulose shows a slight decrease corresponding with the slightly lower glucose values. In treatment C the water content has been fairly steadily maintained at a low value, and the hemicellulose values are correspondingly low except on the last day, when hemicellulose shows a sharp rise. The slight rise in water content seems insufficient to account for this, but there is, however, a correspondingly sharp rise in the glucose concentration with further hydrolysis of the labile, water soluble carbohydrate reserves. Under low light intensity, the water content undergoes more variation. After the initial rise in treatment D the continual rise in water content is not accompanied by a rise in hemicellulose apparently corresponding to the low glucose figures. In treatment E, the glucose value falls below any of the concentrations in the previous treatments and despite a slight increase in the water content the hemicellulose decreases.

Treatment F shows a sharp fall in the glucose concentration and decrease in water content after the initial rise, corresponding with a continued depletion in hemicellulose.

Sucrose shows a drift strikingly paralleled by fructosan. Under treatment A neither sucrose nor fructosan fall to below half their previous

value, whereas depletion is more severe where nitrogen has been added. Under low light intensity the sucrose concentration is depleted to lower values than those shown in treatments A, B and C, corresponding to the lower glucose figures and closely paralleled by the drift in fructosan.

C. Treatment effects.

The effect of increasing amounts of ammonium sulphate to the roots of the plants is shown in fig. 3.

EFFECT OF TREATMENT WITH ADDITIONAL NITROGEN.

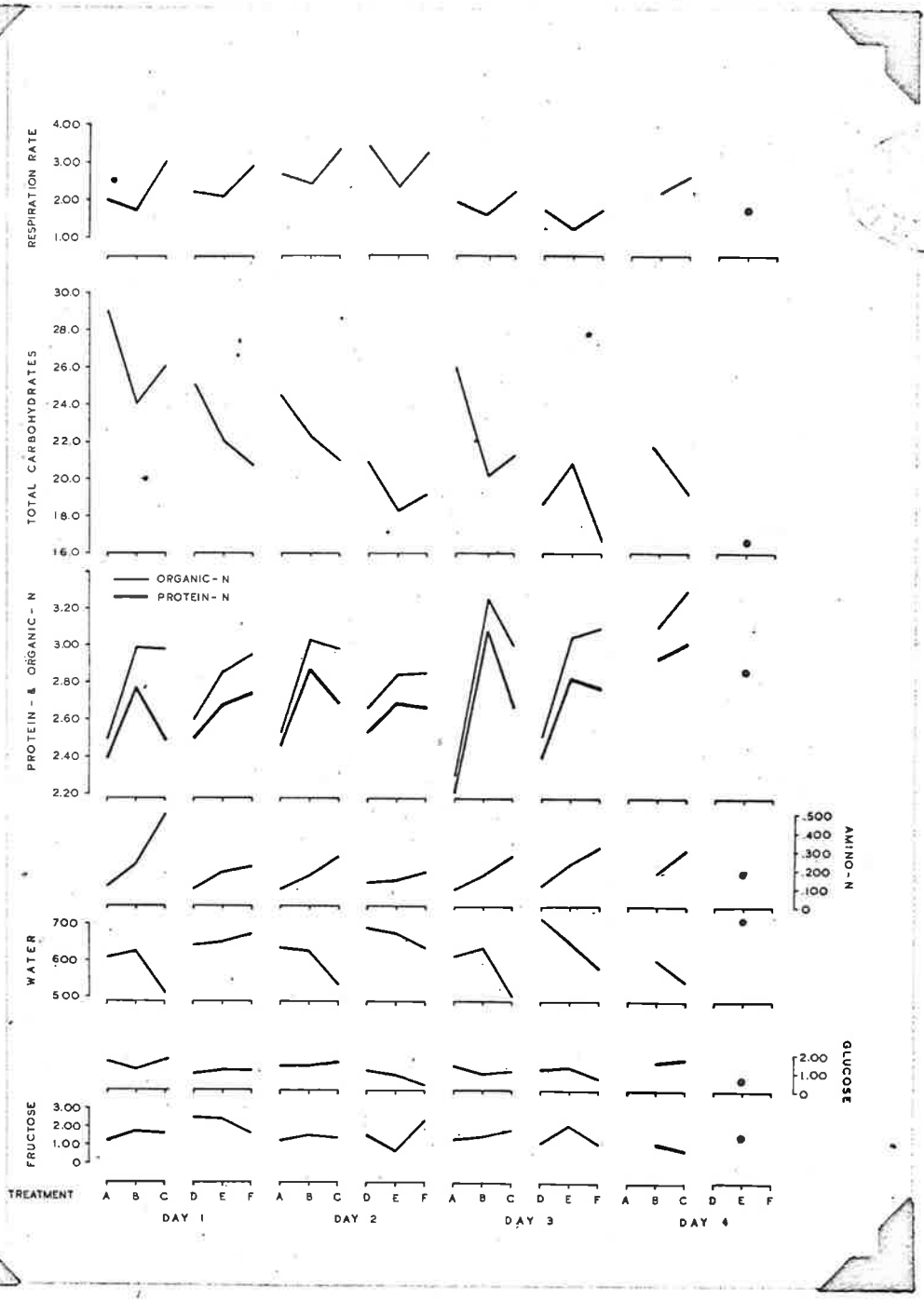


Fig. 3. Treatments A and D no additional nitrogen.
" B " E 2 grms " ammonium sulphate.
" C " F 6 " " " " per pot.

The respiration rate shows a tendency to decrease with the first application and to increase with the second.

The total carbohydrate shows in general a decrease with added nitrogen, treatments A and D always having a higher carbohydrate content than the corresponding treatments under low light intensity. The general behaviour tends to be inverse to that of the organic nitrogen.

Protein and organic nitrogen are much more influenced by the first addition of ammonium sulphate than by the second, the initial rise being more marked under high light intensity than under low, and becoming greater on successive days. Presumably more energy is available for synthesis on these days. The difference produced by the second addition is scarcely significant. The amino-nitrogen, however continues increasing proportionally with the increase in concentration of ammonium ions in the external medium, the increase being greater with the second addition than with the first.

The water content presents a characteristic behaviour under high light intensity, remaining almost constant with the first addition and falling rapidly with the second, and shows higher values under low than under high light levels.

The concentrations of the individual hexoses are little affected by treatment, the noticeable feature being that the glucose concentration falls to a very low value under low light intensity on days 2, 3 and 4, which, as will be seen, is also a characteristic of the sucrose concentration. The low values are more evident under conditions of high nitrogen treatment.

D. Effect of Nitrogen Treatment on the Reserve Carbohydrates.

The effect of added nitrogen on the individual di - and poly-saccharides is illustrated in figure 4.

EFFECT OF TREATMENT ON RESERVE CARBOHYDRATES.

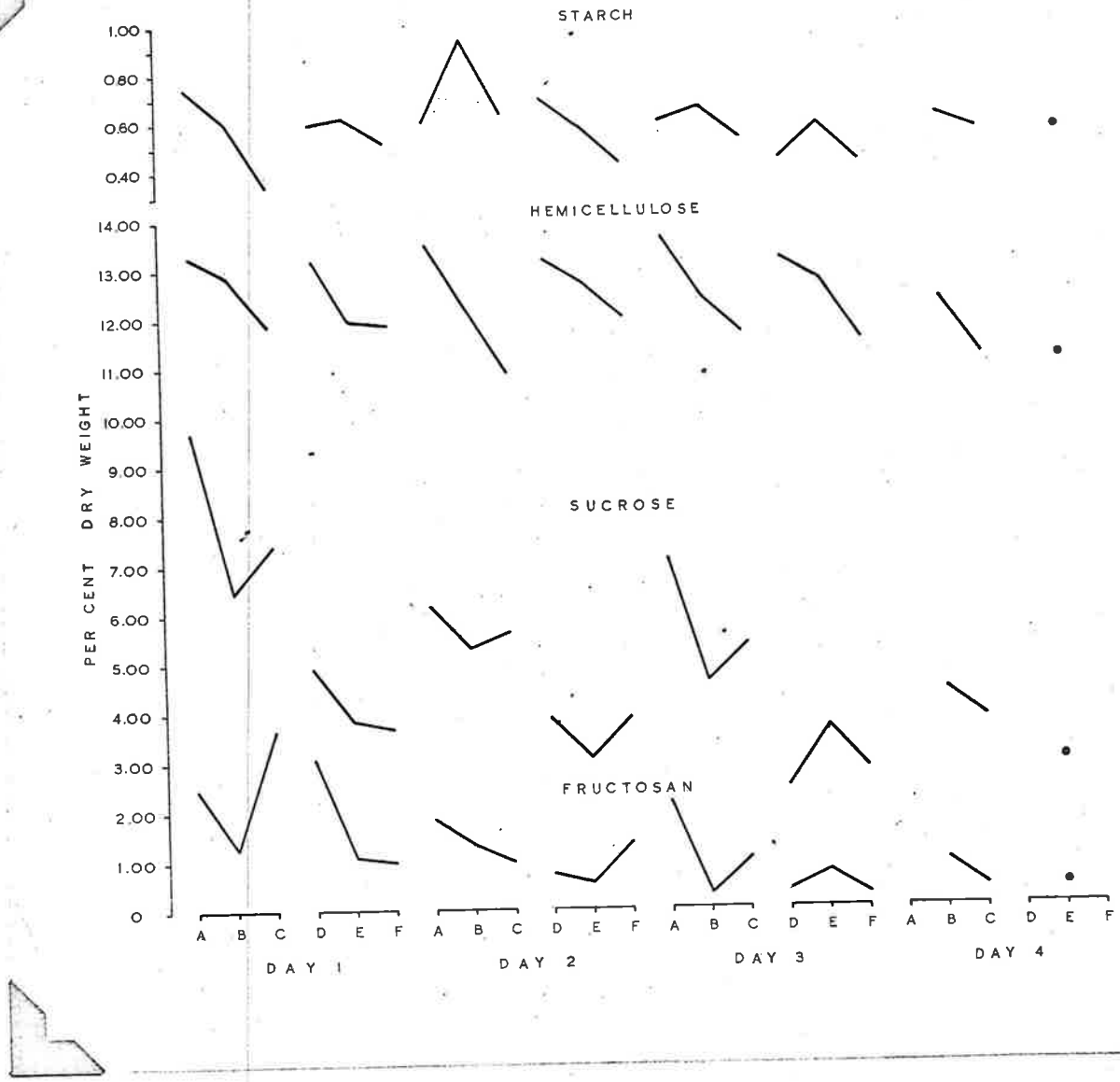


Fig. 4. Treatments A and D No additional nitrogen.
" B and E 2 grms. ammonium sulphate per pot.
" C and F 6 " " " " per pot.
Ordinates represent percentage of dry wt.

No distinct treatment effect on the starch concentration is noticeable.

On all days at both light intensities the hemicellulose is continually reduced by increased nitrogen treatment, and it may be correlated with water and organic nitrogen content. On day 1 under high light intensity the water is constant between the A and B treatments, and is lower in treatment C. The hemicellulose shows a reduction in the B treatment corresponding to a rise in organic nitrogen, and a further fall in treatment C where the organic nitrogen remains constant, but the water content is reduced. The same principles apply on all days under high light. On day 1 under low light the hemicellulose falls off between treatments A and B whence organic nitrogen rises and water content remains constant. A further rise in organic nitrogen and water in treatment C are accompanied by little change in hemicellulose. On days 2 and 3 a continued fall in water with treatment under the low light are accompanied by increase of organic nitrogen with the first treatment and continual decrease in hemicellulose with treatment.

Sucrose and fructosan respond in a parallel manner to increasing nitrogen, sucrose values being low when the corresponding glucose values are also low. The sucrose figures are higher under treatments A, B and C than under corresponding treatments D, E and F on the same day.

E. The Effect of Light Intensity.

Light intensity affects the concentrations as shown in fig. 5.

EFFECT OF LIGHT INTENSITY.

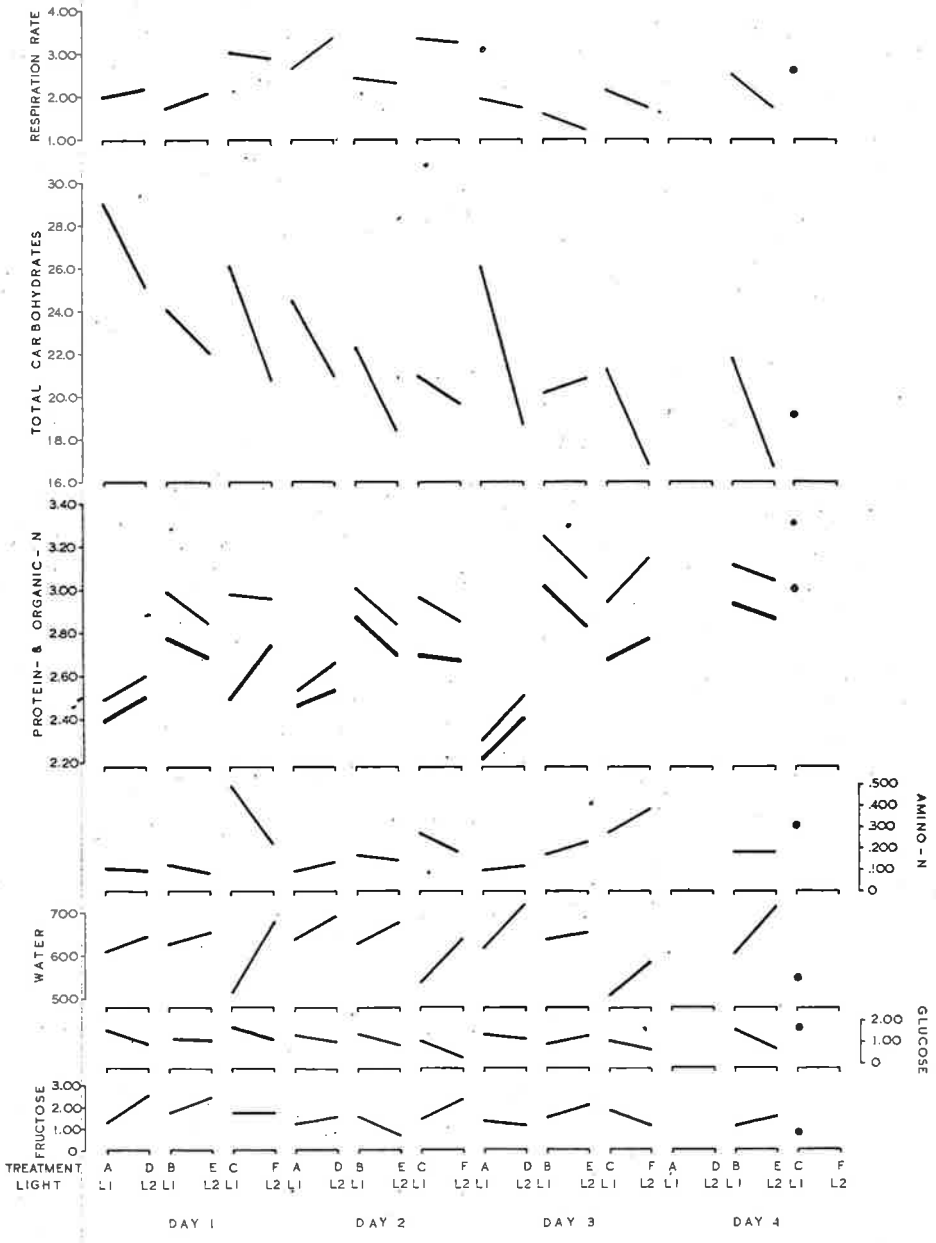


Fig. 5. L_1 = high light intensity.
L_2 = low " "
Corresponding treatments under high and low light intensities are compared on successive days.

In the earlier part of the experiment when the carbohydrate is not severely depleted, light intensity has not affected the respiration rate, but later there is a tendency for lower values to occur under the lower light.

The total carbohydrate is always reduced under the lower light, presumable because of the effect on the photosynthetic system. On one occasion when the respiration rate is a minimum, the carbohydrate content is not significantly altered. The individual carbohydrates will be examined in detail in the next section.

Where no additional nitrogen has been added, organic and protein nitrogen show higher values under the lower light intensity, but in treatments B and E, when one additional quantity of nitrogen has been added, the reverse is the case. With higher nitrogen treatment, the variation is irregular.

Light intensity has had no consistent effect on the concentration of amino-acids.

In all cases water content is higher under low light than under high, the difference being most marked in treatments C and F.

Glucose shows lower values under low light except in one case where the respiration rate is very low, and on that occasion the sucrose shows a minimum depletion under the lower light. Fructose behaves in an irregular manner.

F. Effect of Light Intensity on the Reserve Carbohydrates.

The effect of light intensity on the levels of the reserve carbohydrates is shown in fig. 6.

EFFECT OF LIGHT INTENSITY ON RESERVE CARBOHYDRATES.

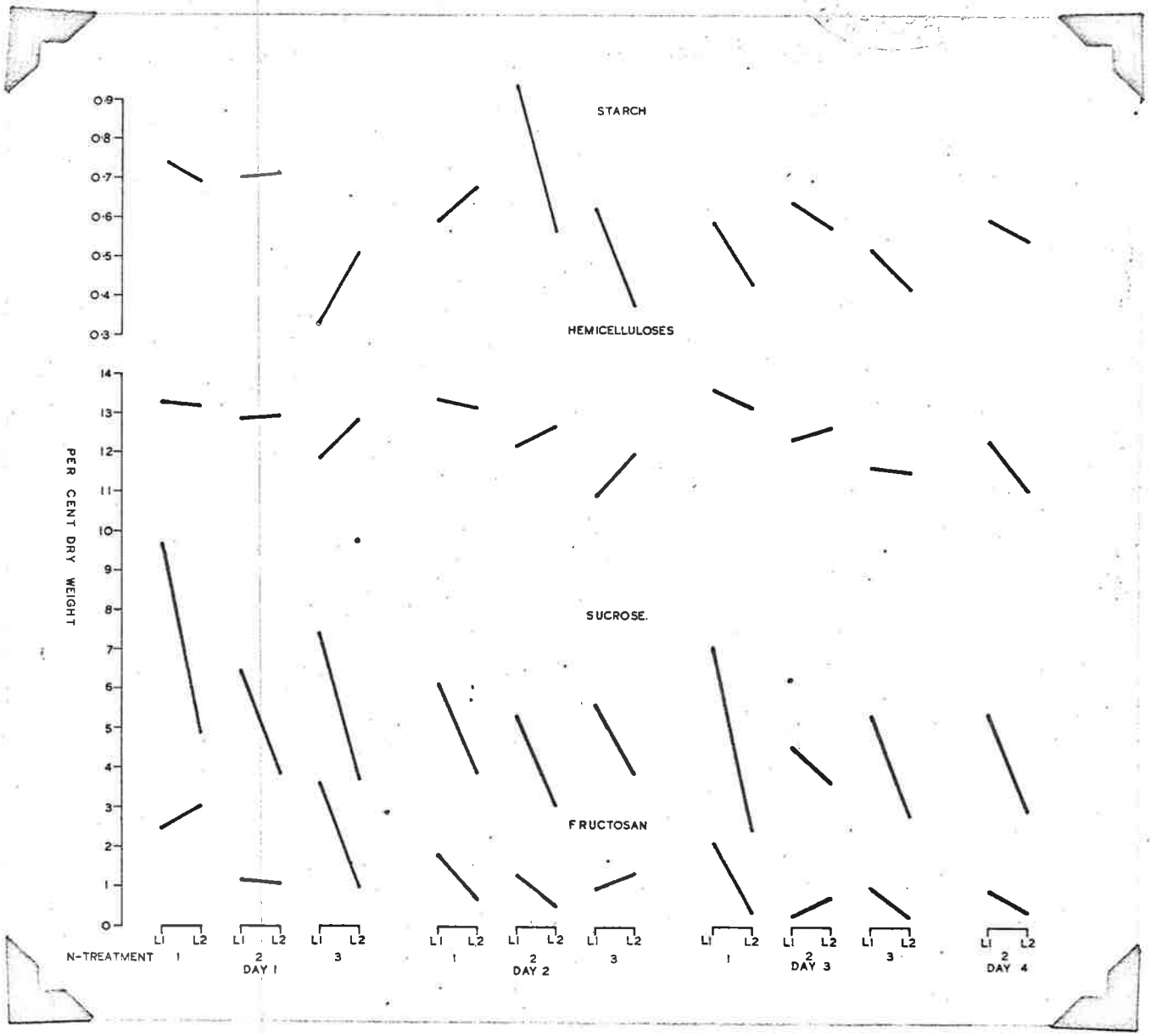


Fig. 6. L_1 = high light intensity.
L_2 = low " "
Corresponding treatments under high and low light intensities are compared on successive days. Ordinates represent percentages of the dry weight.

On the first day the starch figures behave in an irregular manner, but later they assume lower values under the low light intensity. The amount of hemicellulose is also unaffected.

The depletion in total carbohydrate is seen to be chiefly due to depletion of sucrose, the most labile carbohydrate reserve. Consistently throughout the experiment the sucrose concentration has suffered the greatest depletion with reduced intensity of illumination. On day 3 with one lot of additional nitrogen it shows a minimum effect, corresponding to a slight increase in the glucose concentration.

The fructosan, which followed the sucrose in drift and treatment effects, is not so consistently affected.

RELATIONSHIPS BETWEEN CARBOHDYRATE RESERVES, WATER AND ORGANIC NITROGEN.

Sucrose - Water - Organic Nitrogen.

When the sucrose values are plotted against water content and the organic nitrogen figures are included, the distribution shown in figure 7 is obtained.

SUCROSE - WATER - ORGANIC NITROGEN.

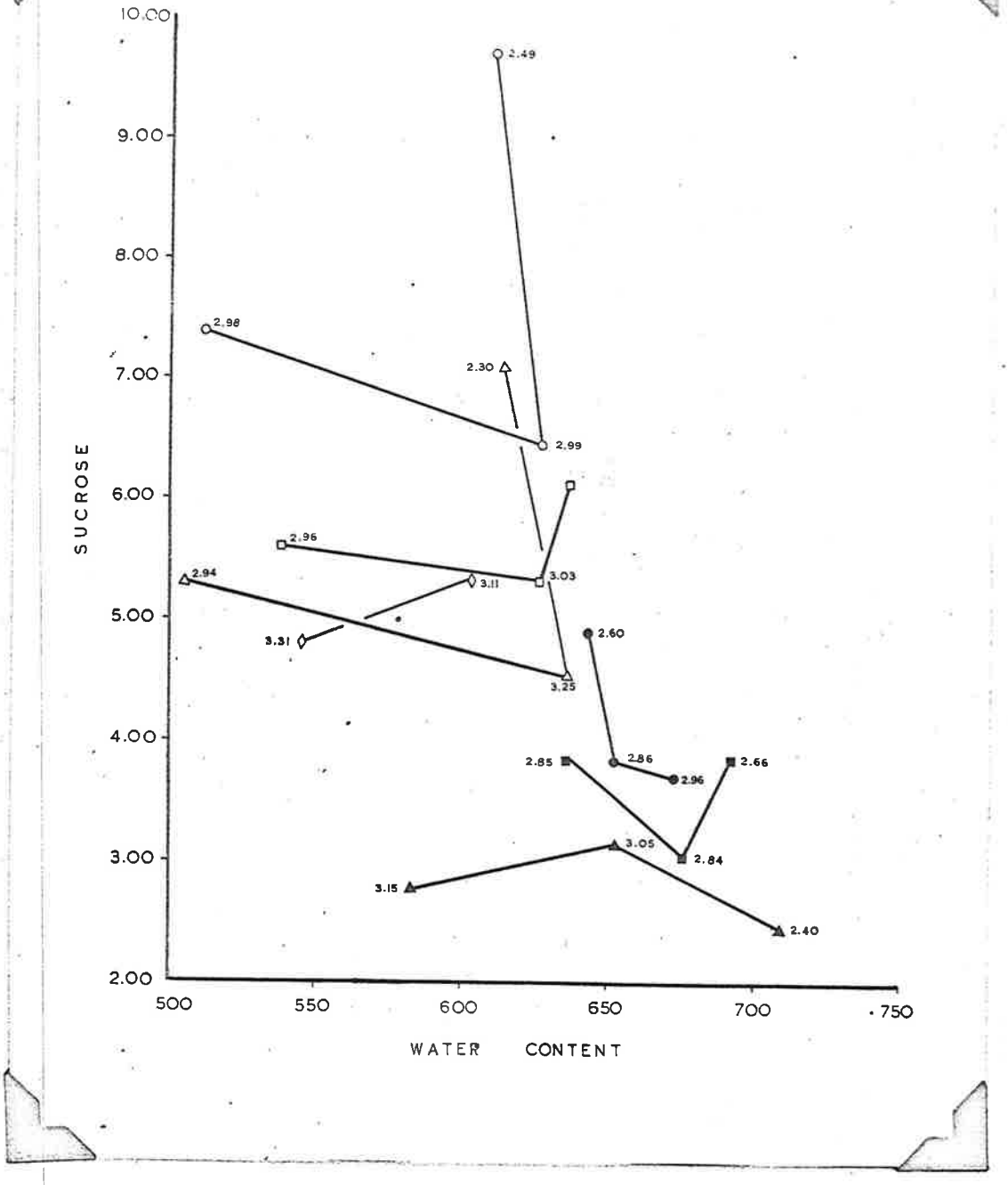


Fig. 7. Relationship between sucrose, water and organic nitrogen.

○ = day 1 high light ● = day 1 low light.
□ = " 2 " " ■ = " 2 " "
△ = " 3 " " ▲ = " 3 " "
◇ = " 4 " "

Sucrose and water as percentage of dry weight
Figures - % dry weight of organic nitrogen.

The figure shows that on day 1 under high light intensity, treatments A and B show very little difference in water content, but sucrose has been reduced and an increase in total organic nitrogen has occurred. In treatment C, the organic nitrogen has reached the same level as in treatment B, but a marked reduction in the water content corresponds to an increase in the sucrose content. On the second day, the sucrose level has been reduced, but the same relationships applies, namely that treatment A has a higher sucrose and a lower organic nitrogen content than treatment B, which in turn has a lower sucrose and a higher water content than treatment C. On the third day the relationship is equally evident. The water content is not significantly altered between treatments A and B, but an increase of about 40% in organic nitrogen is accompanied by a loss of about 40% of the sucrose. An increase in organic nitrogen on the fourth day opposes the effect produced by a difference in water content between treatments B and C. The increase in organic nitrogen has accounted for a greater decrease in sucrose content than can be compensated for by the water difference.

Under low light intensity, the same principles apply. On the first day, an increase in organic nitrogen from 2.60 to 2.96 per cent between treatments D, E and F without any significant variation in the water content is accompanied by a decrease in sucrose from 4.9 to 3.7 per cent. On the second day the difference between treatments D and E results in a higher organic nitrogen and a lower sucrose content in the latter treatment, and reduction in the water content without any change in the organic nitrogen in treatment F is accompanied by a higher sucrose figure. On the third day, decrease in the water content and increase in organic nitrogen act in opposite directions, and the sucrose values do not undergo a very marked variation.

ructosan - Water - Organic Nitrogen

When the same treatment is applied to the fructosan data, fig. 8 is obtained.

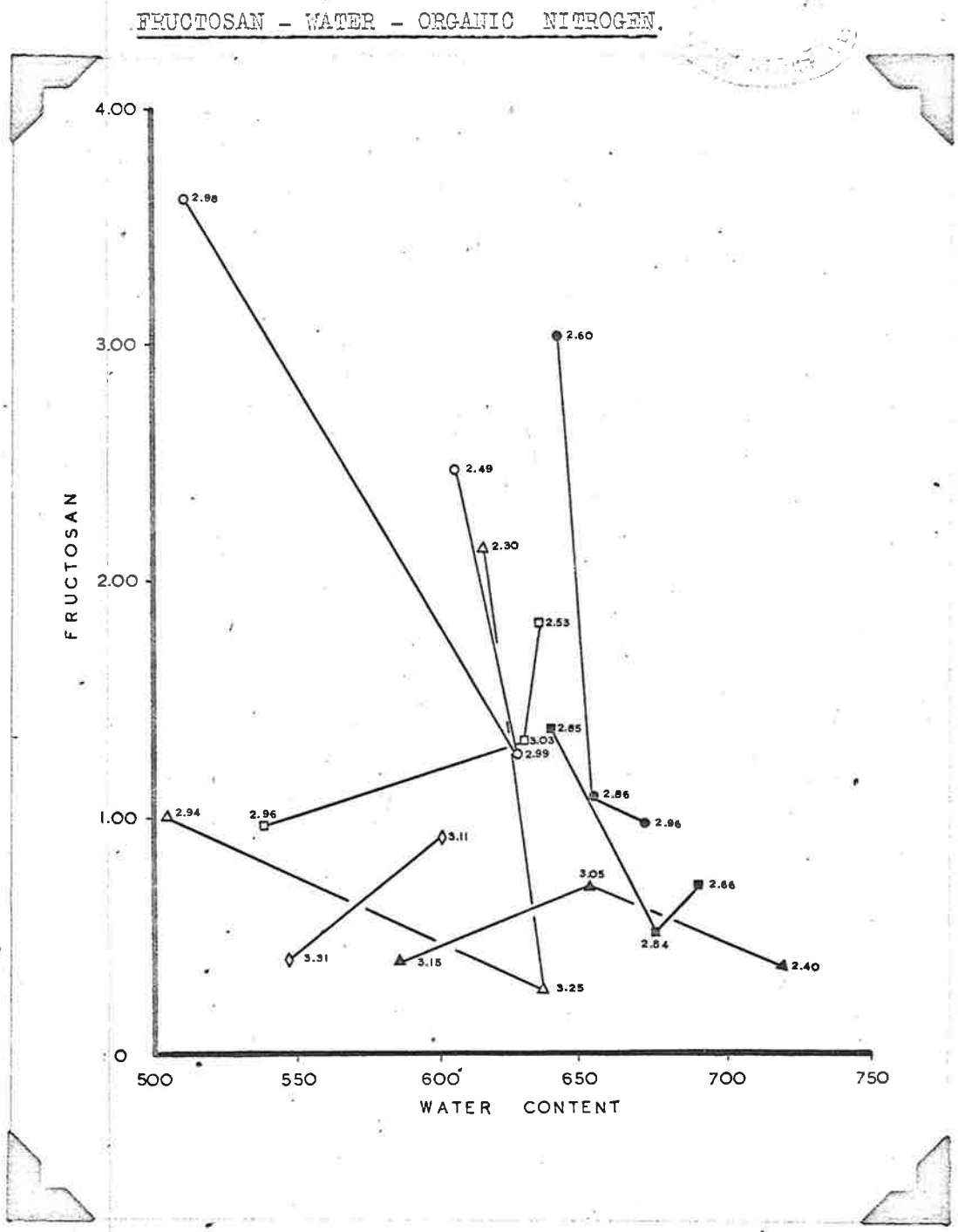


Fig. 8. Relationships between fructosan, water, and organic nitrogen.

Day 1 high light ○ low light. ●
" 2 " " □ " " ■
" 3 " " △ " " ▲
" 4 " " ◇

Figures represent % dry weight of organic nitrogen.

On day 1, under high light intensity, decrease in fructosan and increase in organic nitrogen is the evident difference between treatments A and B, where the water content is not appreciably different. The same difference occurs between these two treatments on days 2 and 3. On days 1 and 3, the organic nitrogen differs very little between treatments B and C, but the water content is markedly reduced, and the fructosan content rises with reduced water. On day 4, the fructosan content falls when the effect of increased organic nitrogen outweighs that of reduced water.

Under low light intensity the same principles apply to fructosan as to sucrose. On the first day an increase in organic nitrogen from 2.60 to 2.96 per cent between treatments D, E and F is accompanied by a decrease in fructosan from 3.02 to 0.97 per cent. On the second day the difference between treatments D and E results in a higher organic nitrogen and a lower fructosan content, and reduction in the water without any change in organic nitrogen in treatment F is accompanied by a higher fructosan figure. On the third day, decrease in water content and increase in organic nitrogen act in opposite directions, and the fructosan figures do not undergo a very distinct variation.

5. Hemicellulose-Water - Organic Nitrogen.

When the amounts of hemicellulose are plotted against water content and the organic nitrogen figures placed at corresponding points, fig. 9. is obtained.

HEMICELLULOSE - WATER - ORGANIC NITROGEN.

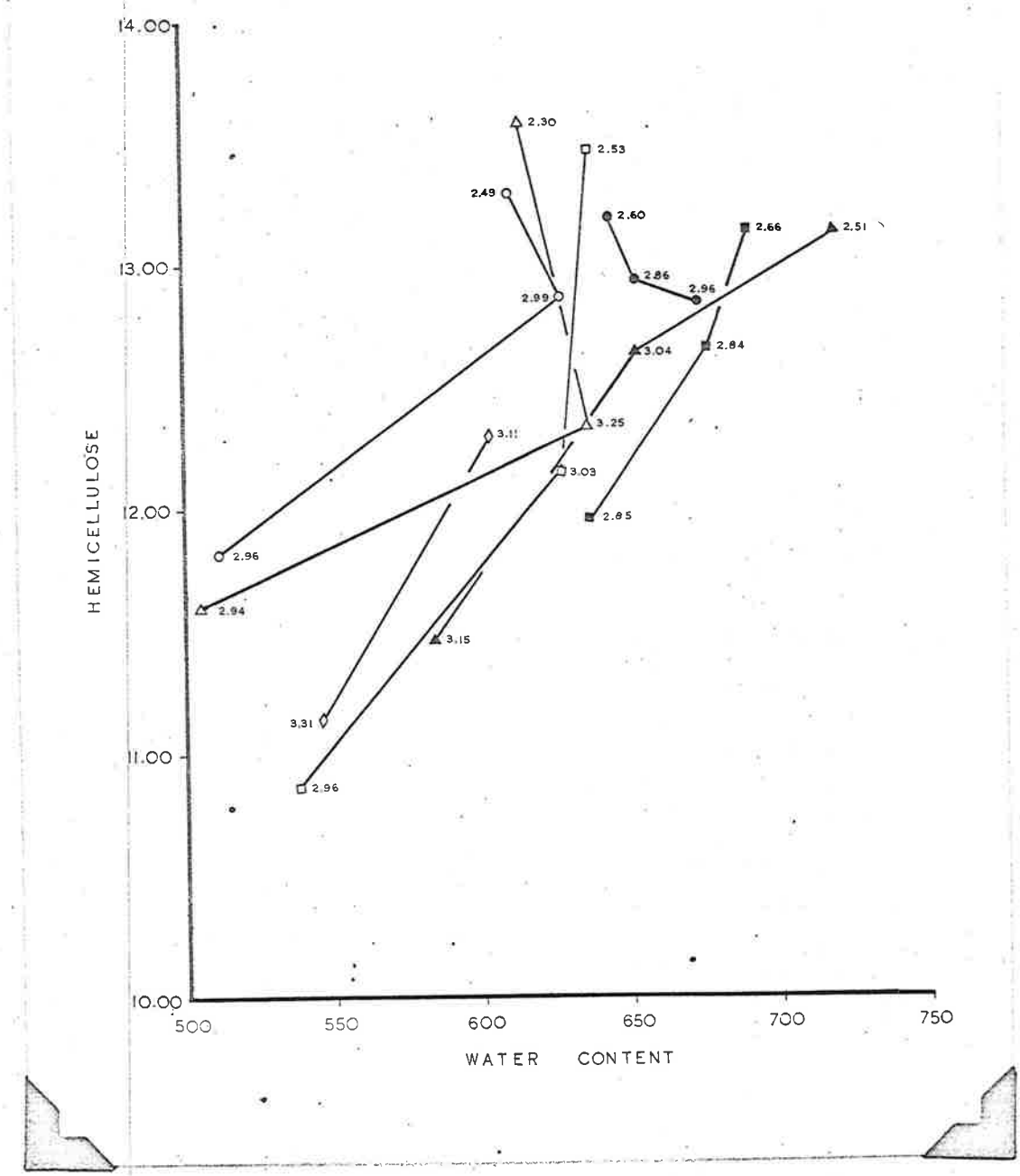


Fig. 9. Relationships between hemicellulose, water and organic nitrogen.

Day	1	high light	○	low light	●
"	2	"	"	□	"	"	■
"	3	"	"	△	"	"	▲
"	4	"	"	◇		

Figures represent % dry weight of organic nitrogen.

Under high light intensity on day 1 the higher organic nitrogen in treatment B is accompanied by a lower hemicellulose content. In treatment C, where the water content is markedly reduced, the hemicellulose content is quite as markedly depleted. On the second, third and fourth day, under high light, the same conditions obtain. The reduced water content thus has the reverse effect on hemicellulose content to what it had on the water soluble reserves.

Under low light intensity the same effect is observed. On the first day, the water content between the treatments was only slight and the differences in hemicellulose content are also small and accounted for by the variation in organic nitrogen. On the second day the water content has a somewhat wider distribution in the different treatments, and the difference in the amount of hemicellulose present is also more marked. On the third day, a larger variation in water content occurs between treatments D and F, and its effect on the hemicellulose content is correspondingly greater, the lower the water content, the lower the hemicellulose. Organic nitrogen and water increase therefore affect the hemicellulose in contrary directions.

It must be borne in mind that the hemicellulose figures have been recorded as glucose equivalents, i.e. as if hemicelluloses were built up exclusively of glucose units. Provided that the composition of Solium hemicelluloses is constant with respect to the relative number of reducing units, the figures are valid for comparative purposes and conclusions based on these comparisons are valid. It is conceivable, however, that factors operating in the metabolic flux would influence one unit more than another, or may affect the composition of the hemicelluloses themselves, in which cases the conclusions involving hemicellulose concentrations would need modification after a more reliable method for hemicellulose estimation is evolved.

tarch - Water - Organic Nitrogen.

The starch figures have been plotted in the same manner as those for hemicellulose and are presented in fig. 10.

STARCH - WATER - ORGANIC NITROGEN.

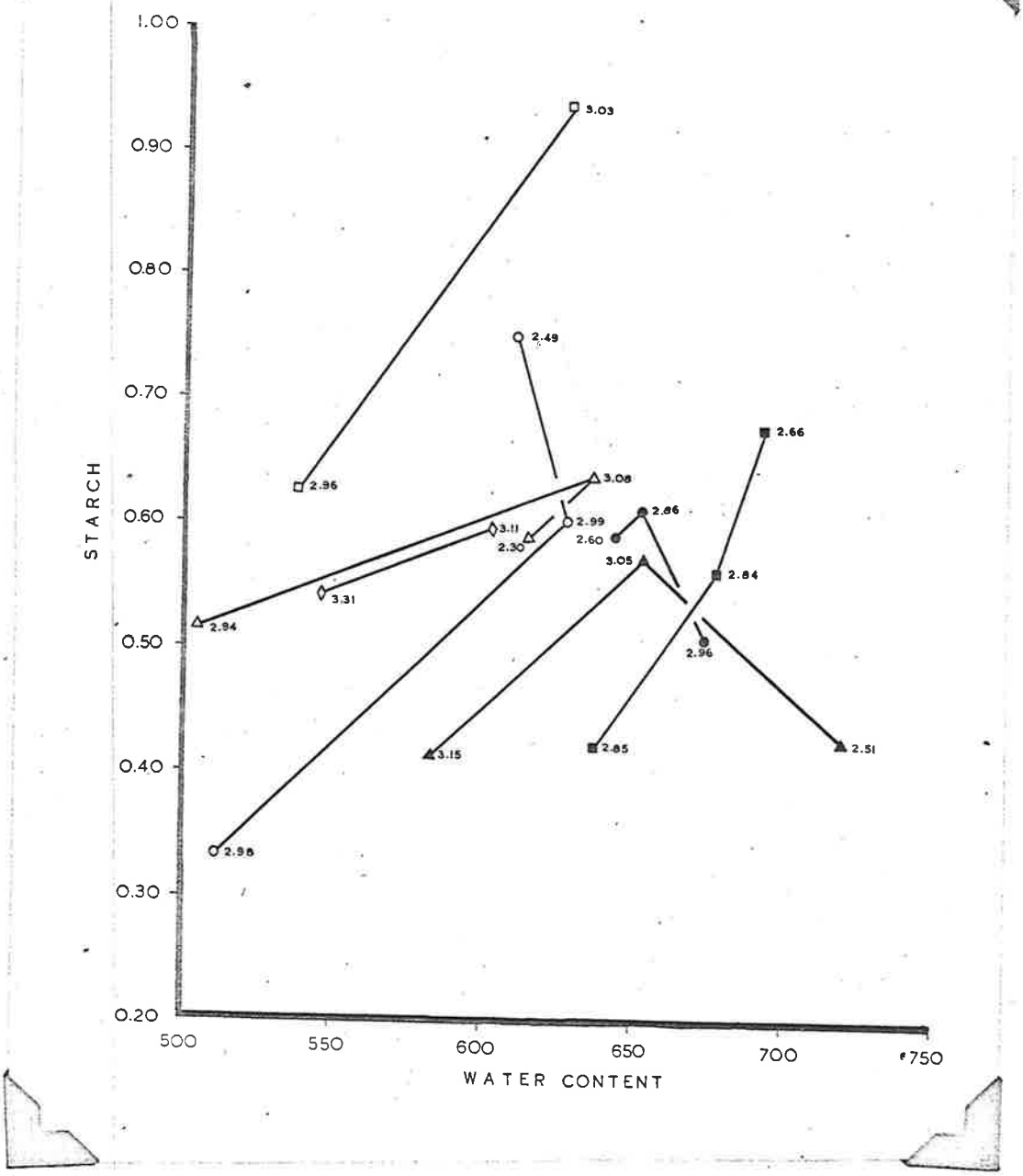


Fig. 10. Relationships between starch, water and organic nitrogen.

Day 1 ' high light low light
" 2 " " " "
" 3 " " " "
" 4 " "

Figures represent % dry weight organic nitrogen.

Owing to difficulties in the method of estimation of this fraction, it is useless to examine the results in greater detail than to look for tendencies. Suffice it then to point out that the same tendencies exist with regard to starch-water relationships as were pointed out in the hemicelluloses, i.e. decrease in water content accompanies decrease in starch and vice versa. The organic nitrogen relationships is not so clearly defined.

D I S C U S S I O N.

rbohydrates.

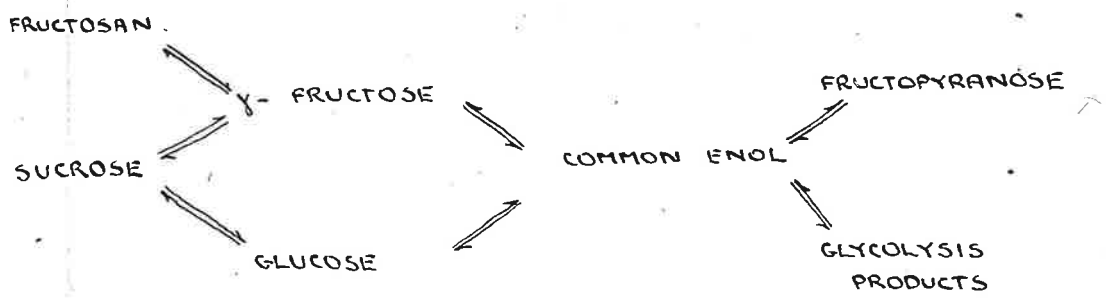
Analysis of the data reveals that a steady state in the metabolic flux has not been attained. The more labile carbohydrate reserve materials, sucrose and fructosan show a rapid depletion during the preliminary period, and this is reflected in the drift of the total carbohydrate. Depletion thence becomes more gradual. The cabinet conditions were therefore not so favourable for carbohydrate synthesis as was the previous environment, and respiration must have accounted for a greater loss of carbon than the photosynthetic system was capable of building up.

The concentration of hexose sugar has not been materially affected by light intensity presumably because, as these have been utilised in respiration or synthesis, they have been simultaneously replenished from the hydrolysis of the more labile water soluble reserves, but where the depletion of these latter is severe, as under the conditions of low light intensity and increased organic nitrogen synthesis the concentration of glucose falls to lower values. This effect is not so marked in the case of fructose, since it is augmented directly from the hydrolysis of both of these reserves, whereas glucose is only directly supplied from one. This accounts for the sharp rise in fructose concentration in all plants on being placed in the cabinets.

On all occasions where the concentration of glucose falls below 1% of the

dry weight, the sucrose concentration has been reduced to below $\frac{1}{3}$ of its former value, and since all these low glucose and correspondingly low sucrose values occur under conditions of low light intensity when photosynthesis is at a minimum, it is evident that a reversible relationship exists between the two sugars. The conclusion to be drawn, since sucrose is a soluble reserve, is that the concentration of sucrose in the leaves is partly determined by the concentration of glucose. The other component liberated on hydrolysis of sucrose would be γ - fructose which would be in equilibrium with glucose through the bridge of the common enol form. The common enol itself would be drawn upon for glycolysis and the normal fructopyranose.

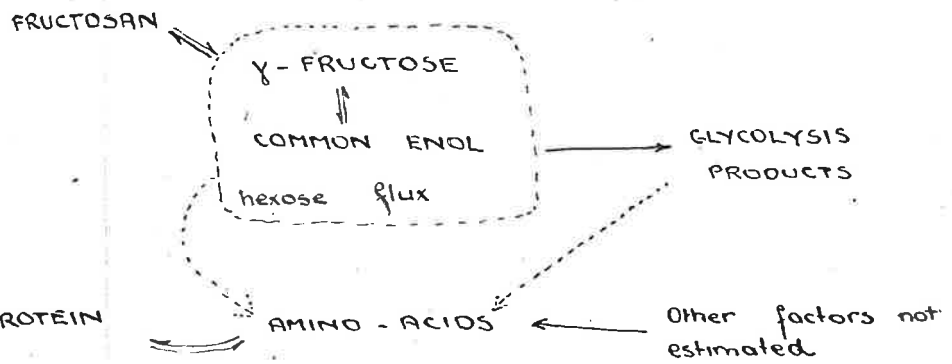
The relationship between these sugars could be represented as follows:-



To further examine the factors determining the concentration of sucrose, it is necessary to obtain some estimate of γ-fructose. The portion of a scheme outlined above would account for the close parallelism in the behaviour of the two-labile water soluble carbohydrate reserves. Indeed a schema of this nature would indicate that the combined concentrations of sucrose and fructosan, or in considering factors determining sucrose concentration, the concentration of fructosan alone, would provide a better estimate of the concentration of the labile fructofuranose than fructose as estimated, since the latter is a mixture of pyranose and furanose forms. Also since the fructosan is independent of the glucose concentration directly its concentration alone is perhaps the surest indication of the amount of γ- fructose. This would explain why the

figures obtained for fructose do not show any apparent relationship to any of the other variables. The parallelism in the drifts of sucrose and fructosan suggests also that a relationship exists between the two, and the fructosan-sucrose-glucose relationship shows a correlation coefficient of .726. Hence sucrose concentration is determined by the concentrations of fructosan and glucose.

Archbold (5) concluded from the results of her work on the role of fructosans in the metabolism of the barley plant that nitrogen deficiency in manurial treatment favoured a high yield of fructosan in plants grown under normal conditions in the field, and that high nitrogen depresses the formation of fructosan. The results of this research confirm her conclusion. If normal conditions are represented by plants growing under the high light intensity in this experiment, the plants with no added nitrogen show a much higher fructosan content than those to which additional nitrogen was added, and after the initial adjustment in concentrations the behaviour of fructosan and organic nitrogen are inverse, a decrease in one being accompanied by an increase in the other. This was pointed out when fructosan was considered in relation to water and organic nitrogen. After the first addition of ammonium sulphate to the roots the organic nitrogen rose and the fructosan concentration fell. Since subsequent mathematical examination shows no reversible relationship to occur between nitrogen and carbohydrate fractions, the following connection is suggested to be added to the scheme



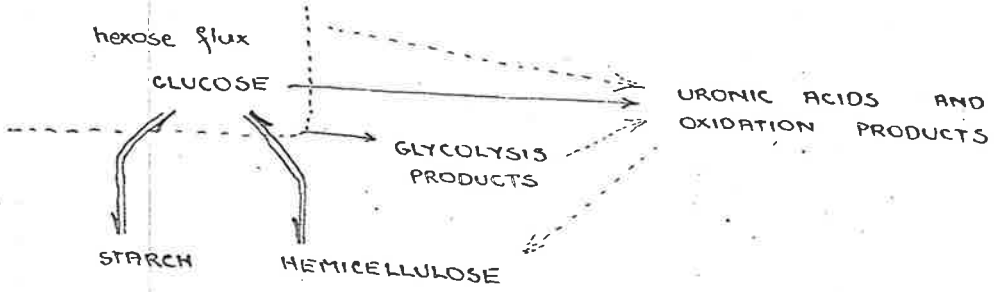
Although no reversible relationship can be shown, obviously the carbohydrate necessary for amino-acid synthesis must be derived from some point in the carbohydrate flux, and with the interplay of so many factors, many of which have not been considered here, involved in a highly complex system in which protein and carbohydrate reserves lie at opposite extremes, trends and tendencies must be considered as important guides to the construction of a schema on which further work should throw added light.

The starch figures are not as reliable as those for the other carbohydrate fractions because of difficulties inherent in the method of estimation. Nevertheless they are depleted with time similar to the soluble carbohydrate reserves. Under low light intensity the depletion is more regular and corresponds to the low glucose concentration. The tendency for starch hydrolysis to accompany decreased water content has also been pointed out, so that both of these factors play some part in the determination of starch content.

The dependence of hemicellulose on water content is much more evident. High water content corresponds to high hemicellulose content and vice versa. In the time drifts where water has shown an initial rise, the hemicellulose has behaved in the same fashion, and in the one case where water did not increase to any extent, the hemicellulose also remained low. It has also been shown that where organic nitrogen increases, i.e. when there is a demand on the carbohydrate system by the nitrogen flux, the hemicellulose content is diminished as well as the other carbohydrate reserves.

The correlated behaviour of hemicellulose, glucose and water has been presented. Since other simple carbohydrates besides glucose are involved in the building up of hemicelluloses, the correspondence in the behaviour of these three variables is remarkable striking. This research has furnished evidence that the hemicelluloses are much more labile than has previously been realized

and play an important role in the carbohydrate balance sheet of the plant. A portion of the scheme to fit the observed facts is as follows:



2. Nitrogen Compounds.

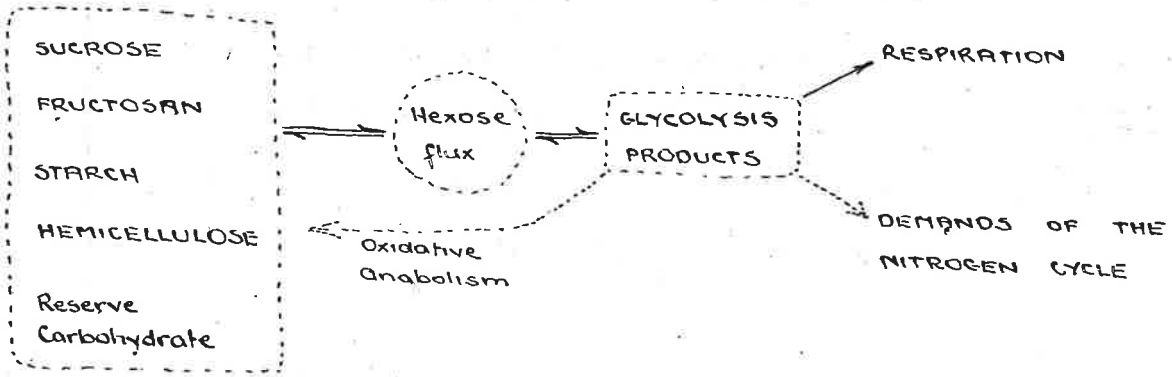
In this experiment only proteins and amino-acids have been estimated. A steady state has not been reached, but in the approach to it, treatment effects have been particularly marked.

The concentration of amino-acids increases with increased addition of ammonium salts in the nutrient medium, whereas protein nitrogen increases with the first addition but shows no further rise with the second. A consideration of the drifts shows that at the end of the experimental period, the total organic nitrogen in the case of themore heavily treated plants was increasing fairly rapidly whereas those with the lower added nitrogen were more steady. However, depletion of the carbohydrate resources is certainly the main factor in limiting the level of the organic nitrogen, since total carbohydrate is more severely reduced in the nitrogen treated than the treated plants. The drifts of protein and total carbohydrate are also in an inverse manner.

Analysis of the individual carbohydrate drifts in an endeavour to locate which is responsible for the inverse behaviour reveals that although in several instances these show a falling off with increased organic nitrogen, no one carbohydrate is closely correlated with nitrogen. It was shown in a consideration of the relationships between carbohydrates,

water, and organic nitrogen that a tendency to become depleted as synthesis of organic nitrogen progressed was evident, but that all carbohydrate reserves contributed a portion.

Gregory and Sen (4) suggested a schema linking the carbohydrate and nitrogen metabolic cycles through the 3 - carbon compounds resulting from glycolysis. According to their schema the hexose concentration determines the rate of formation of glycolysis products. The depletion of these would be a function of oxidative anabolism (Blackman), respiration, and the demands of the protein cycle. The experimental evidence presented here is of a confirmatory nature. Since all the reserve carbohydrates contribute to the hexose concentration in the plant, the reflection of the total carbohydrate rather than individual members is to be expected in the concentration of total organic nitrogen.

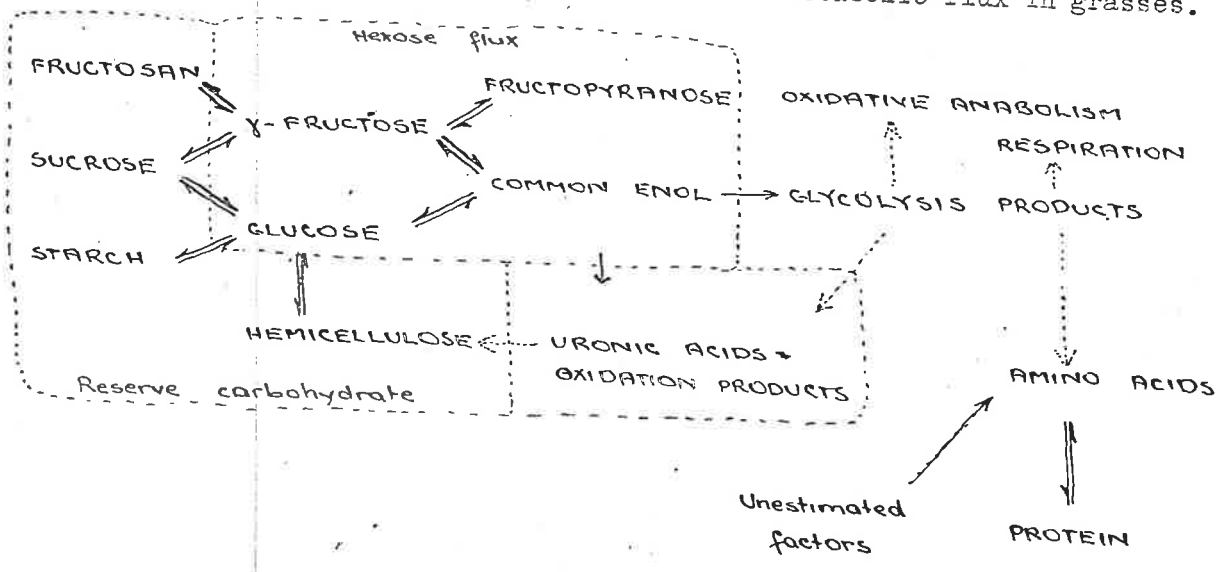


Organic nitrogen supply has been shown to increase when the carbohydrate reserve fell away, water content affecting the soluble and insoluble reserves in an inverse manner, decreased water content favouring decrease, i.e. hydrolysis, of the insoluble reserves and synthesis of the soluble ones.

Finally attention might be drawn to one other tendency. It was shown by Petrie and Wood.(10) that the rate of formation of protein from amino-acids decreases with reduced water content. Now it has been pointed out that the

second increment of ammonia to the roots has not produced a corresponding increment in organic nitrogen, but that at both light intensities, the plants with the higher nitrogen treatment are still showing a rapid increase in this fraction at the conclusion of the experiment whereas those with the lower treatment are decreasing in one case and steady in the other. Now in treatment C, the water content has remained consistently low, the amino-acids have considerably increased, and the protein values are at a lower level than with treatment B, thus indicating thatprotein synthesis has been delayed even though the amino nitrogen level is high. As previously shown, the lack of carbohydrate starting material does account to some extent for the failure of organic nitrogen to reach higher levels with higher treatment, but in this case both factors probably contribute. Petrie and Wood had a much wider variation in water content on which to base their conclusion, but this point of behaviour in this experiment is explicable by their results. The lack of carbohydrate is much more evident in treatment F, where it is continually and rapidly depleted, much more so than in treatment C, but here also, after the initial rise, the water content continually falls, which would tend to delay protein synthesis, even though the amino-nitrogen is slightly higher in concentration.

The following schema is presented for the metabolic flux in grasses.



SUMMARY.

1. A more complete scheme for the estimation of the carbohydrate fractions in grasses than has hitherto been presented has been devised.

2. The loss of carbon has been greater than its replacement i.e. respiration > photosynthesis.

3. The concentration of sucrose is determined by the concentrations of glucose and fructosan. The concentration of fructosan provides the best measure of the concentration of γ- fructose in the leaves.

4. Increase in nitrogen synthesis causes a depletion of all the carbohydrate reserves, which provide separate contributions to the hexose flux.

5. Decrease in water content causes hydrolysis of the insoluble and increase in the soluble carbohydrate reserves.

6. The amount of hemicellulose is determined by the concentration of glucose and the water content.

7. Organic nitrogen has increased after the first addition of ammonium salt but not after the second, partly due to depletion of carbohydrate, and partly possibly, because of decreased rate of protein synthesis under lower water conditions. Amino-nitrogen increases with treatment.

8. A schema is presented for the relations between the various fractions estimated, and their role in the metabolic flux.

A C K N O W L E D G E M E N T S.

I am greatly indebted to Prof. J.G. Wood for suggesting the experiment and conducting a mathematical analysis of the data, to Dr. A.H.K. Petrie for putting his laboratory and apparatus at my disposal, and to the University of Adelaide, who granted me a research scholarship from the Federal Government Grant to Universities.

BIBLIOGRAPHY.

1. KERR, H.W. — Cane Growers Quarterly Bulletin i,133,1934
2. ORCUTT and WILSON — Soil Science 39, 289, 1935
3. GREGORY, F.G. and BAPTISTE, E.C.D. — Ann.Bot. 50, 579, 1936.
4. GREGORY, F.G. and SEN P.K. — Ann.Bot. N.S. 1, 183. 1937.
5. ARCHBOLD, H.K. — Ann.Bot. N.S. 2, 183, 1938.
6. ARCHBOLD, H.K. and BARTER, A.M. — Bioch. Jour. 29, 2689, 1935.
7. ARCHBOLD, H.K. — Ann.Bot. N.S. 2, 403, 1938.
8. PETRIE, A.H.K. and WOOD, J.G. — Ann.Bot. N.S. 2, 33, 1938.
9. WOOD, J.G. and PETRIE, A.H.K. — Ann.Bot. N.S. 2, 729, 1938.
10. PETRIE, A.H.K. and WOOD, J.G. — Ann.Bot. N.S. 2, 887, 1938.
11. BENEDICT, S.R. — Jour. Biol. Chem. 76, 457, 1928.
12. FOLIN, O and SVEDBERG, A. — Jour. Biol. Chem. 70, 405, 1926.
13. HAGEDORN, H.C. and JENSEN, B.N. — Bioch. Zeitschr. 135, 46, 1923.
14. HILLER, A., LINDER, G.C., and VAN SLYKE, D.D. — Jour. Biol. Chem. 64, 625, 1925.
15. SOMOGYI, M — Jour, Biol. Chem. 78, 117, 1928.
16. PETERS and VAN SLYKE — Quantitative Clinical Chemistry, London, 1932.
17. WALKLEY, J. — M.Sc. Thesis, Adelaide.
18. Van der PLANCK — Biochem. Jour. 30, 457, 1936
19. MUNTZ — Compt. Rend. Acad. Sci. 87, 679, 1878.
20. YEMM — Proc. Roy. Soc. Lond. B. 117, 483, 1935.
21. HANES — Biochem. Jour. 36(i), 1936.
22. COLE — Practical Physiological Chemistry 1933, p. 47.