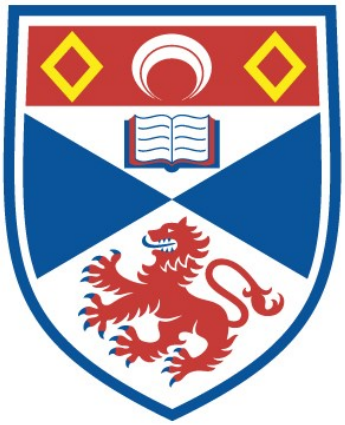
GROWTH AND SOLUBLE CARBOHYDRATE CONTENT IN RELATION TO NUTRIENT SUPPLY : A STUDY OF FOUR GRASS SPECIES

Lynda J. Creedy

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1978

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ABSTRACT

The approach in this study to discover more about the relation between growth rate and adaptation to particular sites was an examination of the quantitative differences in growth, soluble carbohydrate and amino acid content in the four experimental species: Lolium perenne (S24 strain), Dactylis glomerata (S143 strain), Festuca rubra, and Agrostis tenuis. On the basis of the growth studies, the species could be seen to differ and form two groups: L. perenne and D. glomerata in one group, and A. tenuis and F. rubra in the other. When complete nutrient solutions were supplied to these species in treatments of increasing concentration the two groups were seen to differ in the pattern of response of their soluble carbohydrates. When the treatments used in further experiments differed only in nitrate, ammonium, nitrate/ammonium proportions and phosphate at different nitrate concentration, definite treatment effects were found in each species, and in many cases, the response of the species could be seen to be significantly different. Further, in many cases, the differences divided the species into the same two groups observed in the growth experiments. However, when the effects of the separate ions were examined, these species differences appeared to be due mainly to quantitative differences rather than to differences in pattern of response. The examination of amino acid content of the species did not appear to clarify the differences observed between the species, though this examination did explain some of the fluctuations of soluble sugar content which could in some cases be related to the yields and rates of growth in the species.

CERTIFICATE

I hearby certify that Lynda J. Creedy (nee Tuttle) has been engaged upon research from October 1974 onwards under my supervision, to prepare the accompanying thesis for the degree of Doctor of Philosophy.

Signed

Prof. R.M.M. Crawford

St. Andrews
May 1978

DECLARATION

I hereby declare that this thesis has been composed by myself, and that it is a record of work which has been done by myself. This has not been accepted in any previous application for a degree. Any other sources of information have been specifically acknowledged.

Signed

GROWTH AND SOLUBLE CARBOHYDRATE CONTENT IN RELATION TO NUTRIENT SUPPLY:

A Study of Four Grass Species

A thesis presented for the degree of Ph.D at the University of St. Andrews.
1978

by

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STATEMENT

I, Lynda J. Creedy (nee Tuttle), was admitted as a research student of the University of St. Andrews in October, 1974 in accordance with Ordinance General No. 12 and the Resolution of the University Court, 1967, No. 1. The thesis was completed in May, 1978.

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LIST OF ILLUSTRATIONS

ABBREVIATIONS USED

ATP	Adenosine tri-phosphate
conc.	concentration
DMSO	Dimethylsulfoxide
FID	Flame-ionization detector
fig.	figure
g	gram
GLC	Gas-liquid chromatography
HMDS	Hexamethyldisilazan
l	litre
L.S.D.	Least significant difference
me./l	milequivalents per litre
mg	milligram
min.	minute
ml	millilitre
mM	millimole
mM/l	millimoles per litre
NAD	Nicotinimide adenine dinucleotide
NADP	Nicotinimide adenine dinucleotide phosphate
NH_4 -N	Ammonium nitrogen
NO_3 -N	Nitrate-nitrogen
nm	nanometre
ppm	parts per million
TMCS	Trimethylcholorosilane
TMS	Trimethylsilyl

ul	microlitre
uM	micromole

CHAPTER 1

INTRODUCTION

Although the solutions to many ecological problems depend ultimately upon critical portions of the life cycles of species or upon critical environmental conditions, any of which may ultimately determine the success or failure of a particular species in given circumstances, the answers to other problems depend mainly upon the differential quantitative responses of different species to a given environment. Indeed, the major energy releasing metabolic pathways are common to all plants, yet these organisms are very diverse in spite of these metabolic similarities.

This suggests two things. First, in most processes there is an optimum solution for maximum efficiency. Secondly, and very significantly, quantitative differences in species response may be more important in indicating ecological adaptations of species than qualitative differences. Unfortunately, there are limited opportunities for making quantitative comparisons between the behaviour of different species as such studies have in the past been relatively few.

The close relationship between the processes of

plant growth and the physical environment is well known. As said before, many of the unsolved mysteries of plant growth in relation to ecology cannot be explained unless the actions of particular factors influencing specific aspects of growth can be identified. Furthermore, the importance of these factors and their influences must be evaluated quantitatively to determine the relative importance of each effect. This study is an exercise in the measurement of plant growth in relation to nutrient supply.

Much evidence exists which demonstrates differences in the nutrient requirements and responses to nutrients of different species in the field. Many patterns are found which range from complete intolerance of some species to some elements to near dependence. The response of species may be less reduced by low levels of some nutrients than other species or more tolerant of high levels; they may respond at different rates, or the ultimate degree of response may be variable. As a result of these diverse responses of plant species to their environments, one can never be justified in assuming that because two species are growing together in the same environment, the particular conditions of which result in optimum growth

for one of the species, the other species will also show an optimum response.

There may be very large differences in the amounts of dry matter produced by different species. It has been suggested that if such differences in response are maintained over a wide range of nutrient levels and in the absence of interspecific competition, this indicates that such differences are characteristic of the species and inherent in their genetic make-up, and that perhaps there is adaptation to a particular environment (Higgs and James, 1969).

Although some work in this area has involved studies of different species, far more has been done studying ecotypes - plants of the same species adapted to a particular habitat. Anotonovics and co-workers (1967) showed that ecotypes varying in their response to mineral nutrients are often found on soils differing in fertility. Futhermore, ecotypes may differ in their ability to take up nutrients (Snaydon and Bradshaw, 1961). As an example, Snaydon and Bradshaw showed that the ability of Festuca ovina races to take up calcium ions from sites low in concentrations of this ion explained their success on acid soils. Others have shown that high-yielding ecotypes frequently inhabit nutrient-rich habitats (Goodman, 1969). A special case of this last example is that of crop plants. It is well

known that species grown as crops have, almost without exception, much higher relative growth rates than species of natural habitats. In addition, many wild species, which may be adapted to extreme environments, reach their optimum relative growth rate at nutrient levels well below those of crop species which may continue to respond to very high nutrient concentrations (Rorison, 1969). The importance of soil-nutrient status as an ecological factor can be demonstrated by the usage of both natural and artificial fertilisers on grasslands and by the evidence that there exists a wide variety of species whose distribution can be related to soil type.

It is often supposed that a given species will have a competitive advantage over any other species whose productivity is less than its own. However, as the results of experiments attempting to use the effects of a given nutrient treatment on monocultures to deduce the effects of competition in the field have shown, this is not necessarily true. In many cases of competition, it is the least productive species which is seen to have the greatest competitive ability, even if this is measured solely in terms of survival. This condition is known as the Montgomery effect (Montgomery, 1912). The reasoning behind this is most simply that soils poor in nutrients would be quickly

exhausted if plants inhabiting them had rapid growth rates. This in turn would cause nutrient deficiency in these plants causing eventual reduction of survival capacity. There is nothing new about the idea of suppression of growth in apparently favourable conditions. The most obvious occurrence of this is in the dormancy of buds and perhaps in senescence.

The plants we choose for pasture or food crops are chosen for yield. This practice of selection for increasing growth with the increasing use of fertilisers in the artificial culture methods we use today does not demonstrate this Montgomery effect. The result has been an increase in crop yields. In natural populations, any differences in response (yield etc.) are due to forces of natural selection and therefore are adaptive. The importance of the increased crop productivity to man and to animals in general is self-evident. It is only comaparatively recently, however, that the lack of response to increased nutrient levels of plants from low-nutrient sites has been thought to be of adaptive significance.

However likely this is, the mechanism which could be responsible for the restriction of growth in some species when nutrient levels are high is not known. Because two or more species occupying the same habitat or soil type will have to cope with the same

environmental factors, it is reasonable to assume that there will be both qualitative and quantitative similarities which will enable these species to survive in their environment. Alternatively, species occupying very different habitats may exhibit some differences reflecting their different adaptations.

Groves (1964) studied heath vegetation and found species growing on soils so low in phosphorus and nitrogen that crop plants failed to grow altogether. The growth of these species on these very low nutrient sites was accounted for by Groves basically in four ways, the most obvious of which is perhaps very efficient exploitation of the soil by the roots of these species. Symbiosis with micro-organisms in the root environment which aided nutrient absorption accounted in part for the success of these species as did a very efficient redistribution of compounds from tissues which were senescing to tissues which were active. An increase in the hydrolysis of organic phosphates during the plants maximum growth phase also aided in the survival of these plants. Others have put forward explanations for the success of plants demonstrating the Montgomery effect. These have ranged from the idea that success is due purely and simply to a low growth rate through the perhaps obvious ideas of more efficient uptake and conservation of mineral

nutrients to the possibility that these plants have developed metabolisms which can function at relatively low levels of nutrient supply.

There is as yet very little evidence of the existence of the adaptive mechanisms or, if they are present, the extent to which they operate, but as Higgs and James (1969) pointed out, the presence of any of these alternative mechanisms would not make the effect of a low rate of growth insignificant. Their presence would, in fact, make low growth rates all the more effective in limiting the nutrient demands of the species. So, although the effect has been demonstrated, there is as yet no formulation of what biochemically or physiologically constitutes a species adapted to eutrophic or oligotrophic habitats. It is known, however, that a key stage of amino acid and protein synthesis (and therefore growth) is the interaction between nitrogen utilisation and carbohydrate metabolism. Therefore, an examination of the quantitative differences in carbohydrate production may be helpful in discovering more about the relation between growth rate and adaptation to particular sites. To this end, this study will quantify growth and soluble carbohydrate content of four species. In addition, supplementary information on the total amino acid content will be supplied.

Plant Materials

In order to carry out these investigations, it was first necessary to select certain plants which could be easily manipulated in these experiments and which would conform to the patterns of growth which we would expect of species adapted to oligotrophic and eutrophic environments. Grasses were considered most suitable, as it was necessary to use plants which were small, herbaceous, relatively fast growing and easily handled. Additionally, as the use of solution culture was planned, the ideal species would not react critically to low levels of aeration. The grasses seemed ideal especially as they occur naturally over a wide range of nutrient habitats.

Four species of grasses were used for all of the glasshouse experiments. These were *Dactylis glomerata*, *Lolium perenne*, *Agrostis tenuis* and *Festuca rubra*. These were grown from seeds obtained from Wm. Watts (seedsman) Ltd., Cupar. The seeds of *L. perenne* were those of the fast-growing S24 strain. The S143 strain of *D. glomerata* was used. The ages of the seedlings in all the experiments ranged from 5-7 weeks. As well as being a convenient stage of growth on the basis of practical considerations such as the length of time involved per experiment and the size of the plants, the most sensitive parts of the life cycle of a species are

perhaps its germination and the establishment of the seedling.

Agrostis tenuis Sibth. and Nardus stricta L. were the most likely choices for species adapted to oligotrophic sites. They occur frequently on acidic semi-natural grasslands (Higgs and James, 1969). Agrostis grasslands occur on soils which are more or less base-deficient and usually on the steeper, better-drained sites. Seedlings of N. stricta proved difficult to obtain, and the transplantation of the seedlings which were obtained proved so unsuccessful that they could not be used for experimentation.

Lolium perenne L. and Dactylis glomerata L. were chosen as the species adapted to eutrophic sites. L. perenne is a lowland species not naturally occuring on acidic semi-natural grasslands (Higgs and James 1969). It is an important agricultural species with a wide habitat range. D. glomerata is an indicator species of high available nitrogen and is usually found on fertile soils, usually with clover.

Festuca rubra L. was added as a fourth species when N. stricta was dropped. It is a widespread species and is found frequently on heaths and in hill-and-mountain grasslands (Hubbard, 1968) and has been shown to have a lower maximum potential relative growth rate than A. tenuis (Grime and Hunt, 1975). It

therefore seemed a likely replacement for *N. stricta*.

The method of handling these species before, during and after experimentation will be explained in each chapter along with the method of each experiment in detail.

CHAPTER 2

STUDIES OF GROWTH IN THE EXPERIMENTAL PLANTS

Before further investigations can be discussed, it is first necessary to characterise the growth of the species studied.

There are several ways of characterising growth. The measurement of size of plant or plant part can be complex, but as change of size is usually accompanied by a change of weight, weight can be used as a convenient measure of growth. Dry weight will be used in this study in spite of any differences which may be accompanied by differential salt accumulation.

Growth in Full Nutrient Solution

The first glasshouse experiment was a preliminary one designed to find a suitable range of nutrient levels. Seeds of *L. perenne*, *D. glomerata*, *A. tenuis* and *F. rubra* were germinated in seed trays containing a 1:1 mixture of peat moss and sand. Individuals were removed, weighed and placed in 4 concentrations of a modified Hoagland's solution (Epstein, 1972). Because different levels of this total nutrient solution have different amounts of all the ions present, it would be misleading to identify the various treatments by any one compound. The treatments are therefore identified

in terms of the percentage of a standard which it represents. The concentrations of the treatments were 0.1%, 10%, 50% and 100% of this standard solution. If they were represented in terms of one of the ions, namely NO_3, the levels would be 0.14, 1.4, 7.0 and 14.0 me./l respectively. The standard or 100% solution contained the following compounds:

Macronutrients	mM/l	Element	me./l
KNO_3	6.0	N	16.0
$Ca(NO_3)_2 .4H_2O$	4.0	K	6.0
$NH\ H_2PO_4$	2.0	Ca	4.0
$MgSO_4 . H_2O$	1.0	P	2.0
		S	1.0
		Mg	1.0

Micronutrients	µM/l		
KCl	50.0	Cl	0.05
H_3BO_3	25.0	B	0.075
$MnSO_4 . H_2O$	2.0	Mn	0.004
$ZnSO_4 . 7H_2O$	2.0	Zn	0.004
$CuSO_4 . 5H_2O$	0.5	Cu	0.001
H_2MoO_4	0.5	Mo	0.002
Fe-EDTA	20.0	Fe	0.06

The transplanted plants were supported over the liquid nutrient in cups. This solution was completely renewed twice weekly. At this time, the cups, which had been set out in a random arrangement, were rotated. There were 3 individuals (replicates) of each species at each nutrient level.

The species were harvested 6 weeks later. Individuals were weighed immediately on harvesting. These weights are presented in Table 2.1 in the form of

dry weights found by the conversion of fresh weights based on the average change in weight on drying of 25 individuals of each species. These corrected dry weights are presented in graphical form in Figure 2.1.

Table 2.1: Average increase in dry weight per plant over a 6 week growth period in 4 different concentrations of a modified Hoagland's solution. The dry weights were obtained indirectly by applying a correction factor to the fresh weights. Weights in mg.

% conc. of Hoagland's solution	A.tenuis	F.rubra	L.perenne	D.glomerata
0.1	0.9	2.02	15.28	11.75
10.0	7.12	16.18	71.44	46.78
50.0	10.25	10.17	58.37	30.92
100.0	4.94	10.27	26.87	27.37

Figure 2.1 shows simply the general level of yield of each species over the range of nutrients supplied. It can be seen that F.rubra and A.tenuis both have lower yield in terms of dry weight than either L. perenne or D. glomerata. Since the optimum nutrient level for each species appears to have been reached over this range, these changes in dry weight can be shown in another way. Plotting the percentage reduction from maximum yield removes the effect of different amounts of absolute growth. This is shown in Figure 2.2.

This graph shows that the patterns of growth of

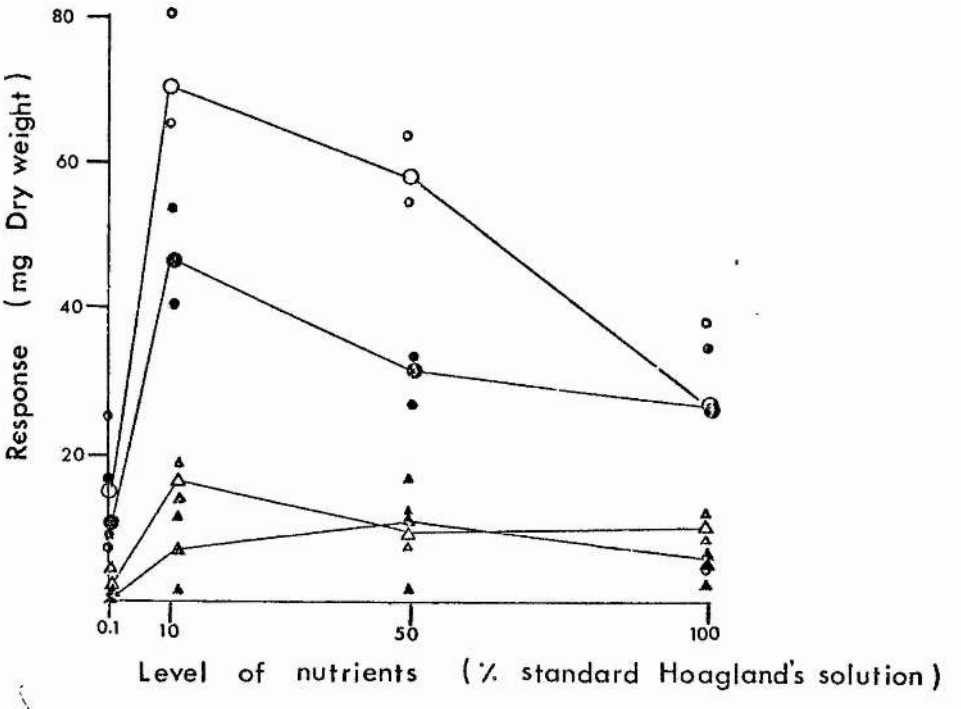


Figure 2.1: Change of dry weights of 4 species after 6 weeks growth in 4 concentrations of a modified Hoagland's solution. Data from Table 2.1. (Data in mg) Values are the means of 3 replicates. The maximum and minimum values of these replicates are also shown

▲ = A. tenuis ● = D. glomerata
△ = F. rubra ○ = L. perenne

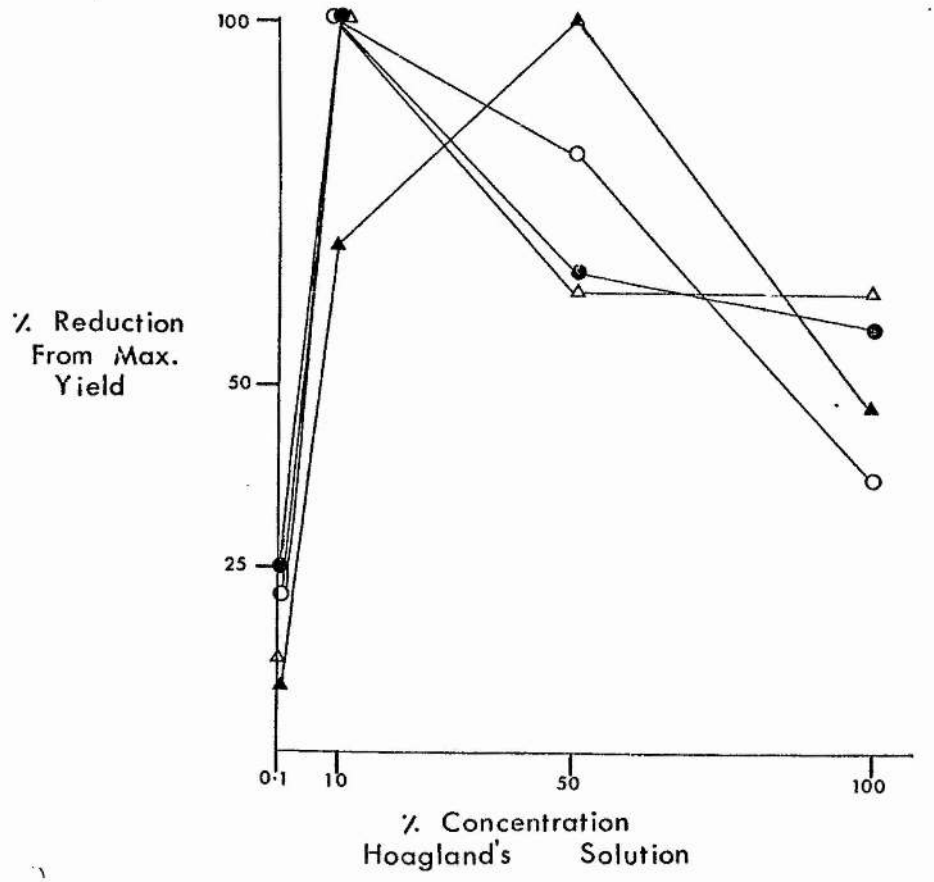


Figure 2.2: Percentage reduction from maximum yield of 4 species grown in 4 concentrations of a modified Hoagland's solution. Yield was base on the average increase in dry weight per plant over a growth period of 6 weeks.

▲= A.tenuis
△= F.rubra
●= D.glomerata
○= L.perenne

the 4 species are more similar than expected. In fact, L. perenne and D. glomerata show a greater reduction from maximum yield than F. rubra at the highest nutrient level. The species of oligotrophic environments, F. rubra and perhaps A.tenuis, have an ability to maintain a steady, although low, production of dry matter over the entire nutrient range. A. tenuis and F. rubra show the greatcst reduction at the lowest nutrient level.

Because these species differ greatly in their actual yield, it is useful to compare them in terms of their proportional rather than their arithmetic differences. Any difference in response of untransformed data could be thought of as representing the actual ecological potential of the species (Antonovics et.al., 1967). Using transformed data, the nature of the response can be detected. Further, assuming an exponential growth rate, the slope of the line obtained when the log / yield is plotted against time will equal the rate of growth, and the Y-intercept will represent the starting capital of the individual.

Table 2.2: Log of the average change in dry weight per plant of 4 species over a 6 week period of growth in 4 different concentrations of a modified Hoagland's solution. This is the data from Table 2.1 transformed logarithmically.

%conc.of Hoagland's solution	A.tenuis	F.rubra	L.perenne	D.glomerata
0.1	1.9542	0.3054	1.184	1.0704
10.0	0.8525	1.2089	1.8539	1.67
50.0	1.0107	1.0072	1.4903	1.4903
100.0	0.6937	1.0115	1.4292	1.4373

Figure 2.3 shows the above data in graphical form. The first point to notice is the general level of yield attained by each of the species. L. perenne and D. glomerata show a high yield, while a lower yield is attained by A.tenuis and F. rubra.

Graphed in this way, parallel lines or segments of lines suggest that either the growth rates or the initial starting capital (on a log scale) has been changed equally in those species by the nutrient treatments. Subsequent plotting of shoot dry weight (log scale) against time (Figures 2.4a-f) showed that a large part of the effect shown in Figure 2.3 was due to different starting weights of the different species at each nutrient level. This was unavoidable due to the method of using seedlings in the experiment. Nevertheless, A. tenuis is seen to have a lower growth

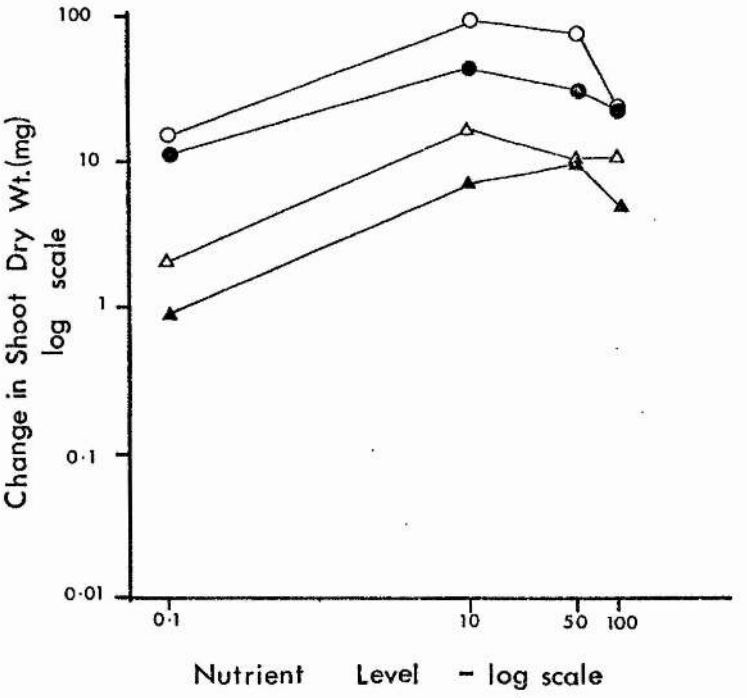


Figure 2.3: Log average change in dry weight per plant of 4 species over 6 weeks. Three individuals of each species were grown in each of 4 different concentrations of a modified Hoagland's solution during this period. Data is from Table 2.2. The original dry weights were in mg.

▲= A.tenuis
△= F.rubra
●= D.glomerata
○= L.perenne

Fig. 2.4a: 0.1% Nutrient Level

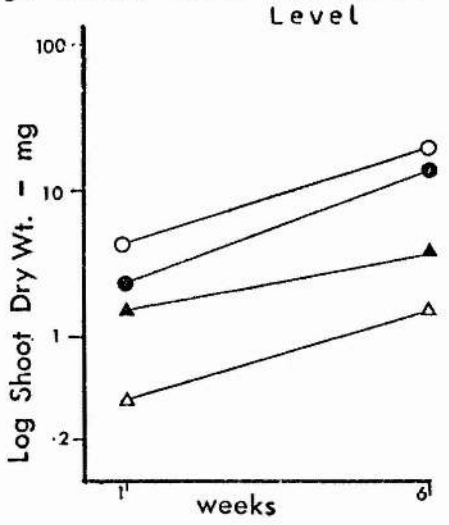


Fig. 2.4b: 10% Nutrient Level

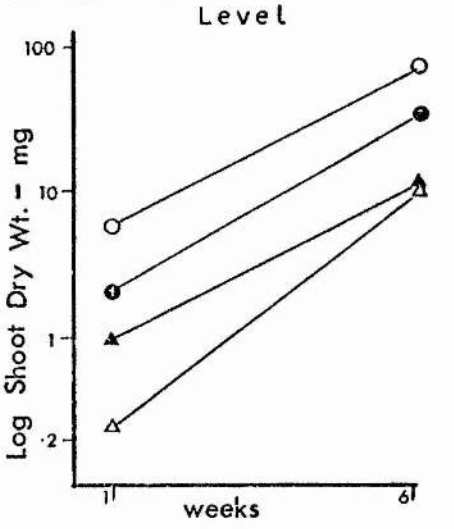


Fig. 2.4c: 50% Nutrient Level

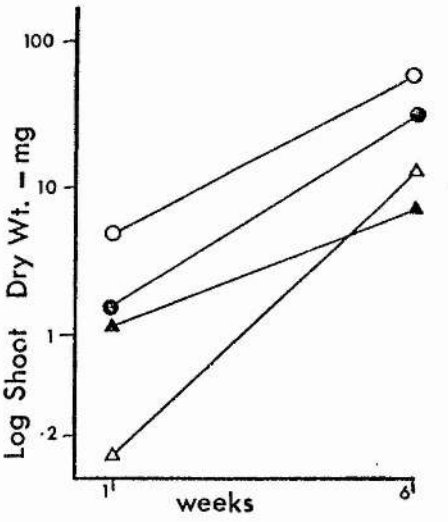
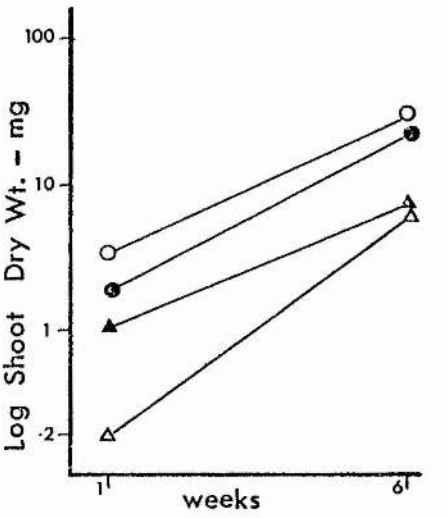


Fig.2.4d: 100% Nutrient Level



Figures 2.4a-d: Change in log shoot dry weight with time of 4 species grown at 4 nutrient levels, based on the average change in dry weight per plant of 3 individuals of each species over 6 weeks.

○= L.perenne, ●= D.glomerata, ▲= A.tenuis and △= F.rubra.

Fig.2.4e: Growth Rate of L.perenne and A.tenuis at 4 Nutrient Levels

Fig. 2.4f: Growth Rate of D.glomerata and F.rubra at 4 Nutrient Levels

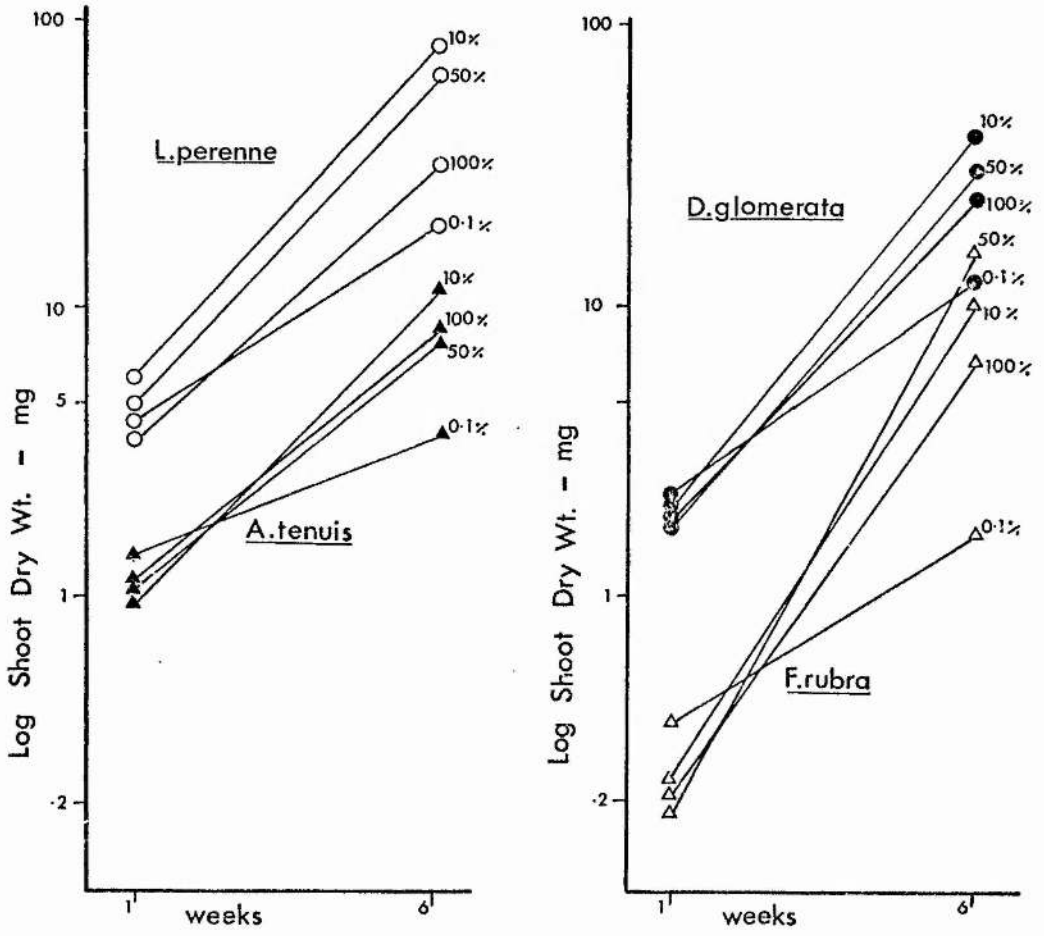


Figure 2.4e-f: Change in log shoot dry weight with time of 4 species grown at 4 nutrient levels based on the average change in dry weight per plant of 3 individuals of each species over 6 weeks. These two graphs show all the treatments of the species together.

rate at all nutrient levels than any of the other species. F. rubra appears to have the greatest growth rate at all but the 0.1% level. At each nutrient level, the order of growth rate, lowest to highest, is A. tenuis, L. perenne, D. glomerata and F. rubra. The order of average amount of stem dry weight at each nutrient level is F. rubra, A. tenuis, D. glomerata and L. perenne again from lowest to highest yields.

If each species is examined separately on these graphs of shoot dry weight (log scale) against time, other trends are apparent. L. perenne shows an increase in growth rate over the 0.1%, 10% and 50% nutrient levels, and a very slight decrease in growth rate at the 100% nutrient level. D. glomerata has a low growth rate at the 0.1% level and reaches its maximum rate of growth at the 10% treatment. The rate then drops at the 50% nutrient treatment, but then at the 100% nutrient level the highest growth rate of this species was reached once again. F. rubra shows a pattern similar to that of L. perenne in that its growth rate appears to increase as the nutrient level increases over the three lowest levels. It then shows a decrease in growth rate at the 100% treatment. A. tenuis has a different pattern. With this species, growth rate is again lowest at the 0.1% treatment and increases at the 10% level (though not as much as the otner species) and decreases

over the 50% and 100% treatments. All these patterns are illustrated in Figures 2.4a-f.

The species vary considerably in their response to variations in nutrient concentration. This is apparent from the results of an analysis of variance performed on the logarithmic values of the dry weights of each species at each of the nutrient levels (Table 2.3). The test used was an analysis of variance for 2 experimental factors without replication (Bishop, 1971).

Table 2.3: Analysis of variance of shoot dry weight (logarithmic values) of the 4 experimental species grown in 4 nutrient levels. The data is from Table 2.2.

Source of Variance	Sum of Squares	d.f.	Mean Sq.	F
Between species	2.0447	3	0.6816	31.9 ***
Between levels	1.2939	3	0.4313	20.2 ***
Residual	0.1926	9	0.0214	

*** $p<0.001$

The variance between species is significant at the 0.1% level. The different species show highly significant differences in the amount of dry matter produced. The between-treatment variance ratio shows that there is also a highly significant difference in the amount of nutrient effect on the dry weight gain. If the 'least significant difference' is calculated, it

can be used to group those means which do not differ significantly (Table 2.4).

Since the average effects of both species differences and nutrient treatements have been found to be significant by the F-test, the significance of the difference between pairs of individual treatment means can be tested using the T-test. The grouping using L.S.D. is only a guide to the values which may be significant, and the procedure can suggest erroneously significant results. The results of a T-test show that on a logarithmic basis A. tenuis and F. rubra are not significantly different in their dry matter production over the nutrient levels used in the experiment; neither are D. glomerata and L. perenne. However, the F. rubra and A. tenuis species are each significantly different from both the D. glomerata and L. perenne species.

Table 2.4: Confidence limits and L.S.D. for the between-species mean values of log change shoot dry weight for 4 species grown at 4 nutrient levels. Change in dry weight is based on the average change in shoot dry weight per plant of 3 individuals of each species over 6 weeks.

Confidence limits: X + 0.2033
L.S.D. (P 0.05) = 0.2875

A. tenuis	F. rubra	D. glomerata	L. perenne
0.6392	0.8833	1.417	1.4894

Bracketed means do not differ significantly

As stated before, a large part of the effect shown in Figure 2.3 may be due to the different starting weights of the seedlings at each nutrient level. To get around this problem of different starting 'capital', it was necessary to use a different experimental method. A second experiment using 9 levels of Hoagland's solution was carried out on the same 4 species. These 9 levels corresponded to 0.1%, 10%, 31%, 68%, 100%, 150%, 200%, 250% and 300% concentration of the basic solution (page 12). This time each species had 4 replicates. A number of seeds of each species were sown on non-absorbent cotton wool in identical plastic pots. The number of seeds per pot for L. perenne, D. glomerata, F. rubra and A. tenuis were 75, 75, 70 and 100 respectively. These numbers were chosen on the basis of the average number of seeds needed to

cover the surface of a pot lightly without overlap of seeds, and so was based on seed size. These pots were then placed in trays of the appropriate nutrient solution. In this way, the plants were watered from the bottom and nutrient solutions could be changed or renewed without much disturbance to the plants themselves. This experiment was done in a glasshouse during March and April 1975. Growth took place over 5 weeks. The trays of nutrient solution and treatment pots were placed in random order, and the trays and the pots in each tray were rotated twice weekly when the solutions were renewed.

Table 2.5: Analysis of variance of shoot dry weight (logarithmic values).

Source of Variance	Sum of Squares	d.f.	Mean Square	F
Species	26.6312	2	13.3156	96.8 ***
Treatment	8.9864	8	1.1233	8.2 ***
Interaction	6.497	16	0.4061	2.9 **
Residual	11.1478	81	0.1376	
Total	50.9698	107		

***= P< 0.001
**= P< 0.01

The shoot dry weight values for the final yield are given in Figure 2.5. Again, the results are expressed in logarithmic values. The percentage reduction from maximum yield is expressed in Figure 2.6. Because of a number of missing values, the data

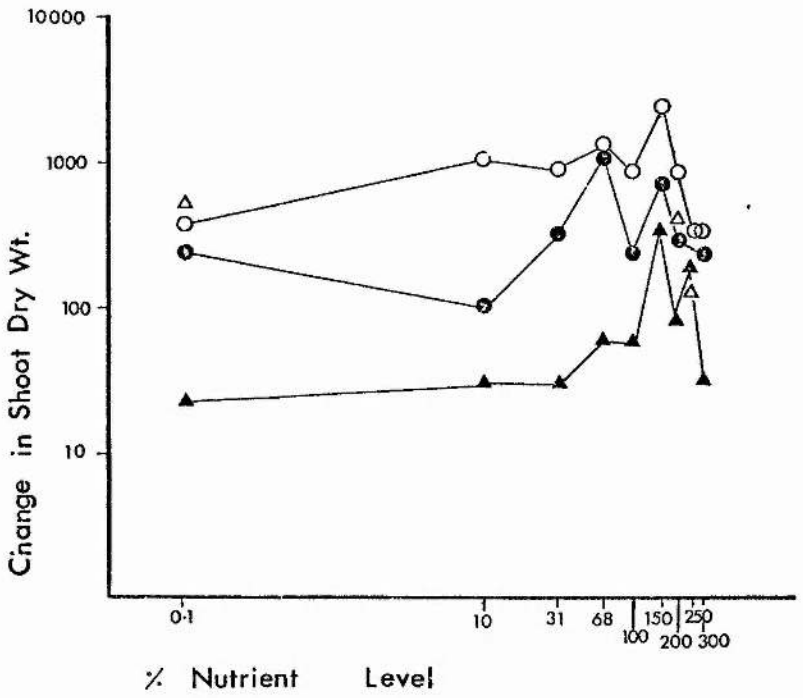


Figure 2.5: Log average increase in shoot dry weight per plant of 4 species over a 5 week growth period in relation to variation in the level of a modified Hoagland's solution. Original dry weights were in mg. Values are the means of 4 replicates. The maximum and minimum values of these replicates are also shown.

▲ = A. tenuis
△ = F. rubra
● = D. glomerata
○ = L. perenne

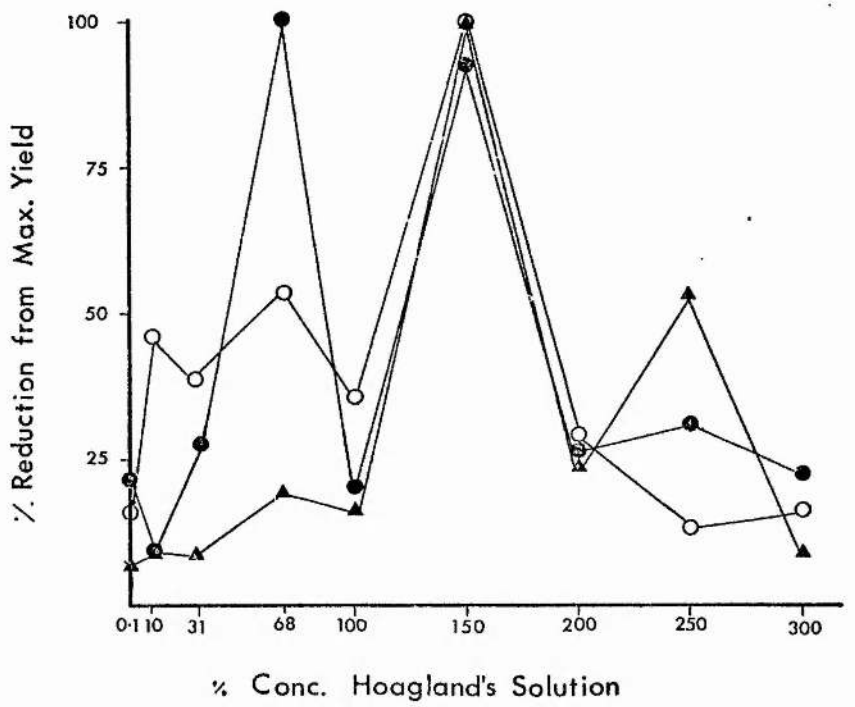


Figure 2.6: Percentage reduction from maximum yield of 3 species grown in 9 concentrations of modified Hoagland's solution. Yield based on the average increase in shoot dry weight per plant over a growth period of 5 weeks. Values are based on the means of 4 replicates.

▲ = A. tenuis ● = D. glomerata
○ = L. perenne

for F. rubra was left out of the statistical analysis. The design of this experiment makes it possible to use a two-factor analysis with replication (Bishop, 1971). This analysis allows the residual mean square to be partitioned into two fractions. One of these represents the mean square for the interaction of the two factors (here, the nutrient level and the species), and the other represents the true residual mean square.

It can be seen from Table 2.5 that both the between-species variance and the between-treatments variance are highly significant ($P<0.001$), duplicating the results of the previous experiment (Table 2.3). In addition, the results of this experiment show that a significant ($P<0.01$) interaction between species and nutrient level occurs. Because an interaction is shown, the data can be broken down into separate tables for each species and even for the nutrient level in order to discover something about the nature of the interaction.

When these separate analyses are in fact carried out, it was found that for each species nutrient level variation had a significant effect: significant at the 1% level for L. perenne and D. glomerata and significant at the 0.1% level for A. tenuis. When the data were broken down by nutrient level to test the effect of species difference at each level, the

following was found: from the 0.1% nutrient level up to and including the 31% level, the species are highly significantly different ($P<0.001$); the three species are significantly different ($P<0.01$) at the 68% nutrient concentration, highly significant ($P<0.001$) at the 100% concentration and marginally significant at the 150% level; the values of the three species at the three highest levels of nutrients (200%, 250%, and 300%) are significant at the 1%, greater than 5%, and 1% levels of probability respectively, the values at the 250% nutrient level not being significant at all. It would then seem that at the lower nutrient levels up to 31% concentration and certainly not over 100% concentration the differences in shoot dry weight among the three species are very probably due to real differences in response to nutrients and not to chance. At the higher concentrations of Hoagland's solution, although some levels show a significant between-species difference, there is a general lower level of significance. This indicates that it is mainly at these lower levels of nutrition that the difference between the species are seen.

Summary of Results of the Full Nutrient Solution Experiments

In terms of actual shoot dry weight, *F. rubra* and

A. tenuis each show a considerably lower yield than either L. perenne or D. glomerata. The four species can be ranked in order of yield, lowest to highest, as follows: A. tenuis, F. rubra, D. glomerata and L. perenne. These species apparently have similar ranges of yield under the conditions of these experiments but their yields at optimum are limited by differences in absolute growth.

At this point something must be said about the apparent differences between the two whole nutrient experiments. The patterns of growth of the four species are similar in both experiments. The differences are that (1) the final shoot dry weight per individual is higher in the first experiment than in the second, and that (2) there is a different position of optimum response for the species in the two experiments, in that the optimum response for each species seems to occur at a lower nutrient level for each species in the first experiment. The first major difference, namely the greater shoot dry weight per individual in the first experiment, is most likely due to a combination of 3 effects. Firstly, the average shoot dry weight in the first experiment is the average of three individuals while the dry weight in the second experiment is the average of 70 to 100 individuals depending on the species. The three individuals in the

first experiment were seedlings when the experiment started and were chosen to be as uniform as possible. The second experiment, however, used many individuals and started from seed and would therefore contain individuals of extremes of yield which would tend to lower the overall average. Additionally, the preliminary experiment used fresh weights converted to dry weights on the basis of average loss in weight of 25 individuals of each species on drying, while the second experiment used actual dry weight. In the first experiment, individuals were grown in separate containers of each nutrient solution, while in the experiment which followed, a large number of individuals shared the container of the same nutrient solution. It is probable that the second experiment shows some element of intraspecies competition, and that the individuals in the first experiment each received more nutrients at a given nutrient level than those corresponding individuals in the second experiment. This may explain both the higher general level of yield per individual in the first experiment and the observation that the optimum response in this experiment occurs at a lower nutrient level.

As the final result in terms of yield can be due to a combination of a number of effects including different starting weights, different amounts of

absolute growth, different growth rates, a difference in the speed of response and a greater or more efficient utilisation of nutrients, a number of ways of handling the data are necessary and helpful in characterising the growth of these species. One of these ways is to plot the percentage reduction from maximum growth. This will remove the effect of different amount of absolute growth and enable the amount of growth to be seen. Thus, the species' response over the given nutrient range is measured as a percentage of the species' best performance. As explained in the text, the patterns seen are more similar than expected in both whole nutrient experiments. This supports the earlier statement that the main difference between the species in this study appears to be a difference in yield at the optimum nutrient level under the conditions of the experiments. It has been suggested (Antonovics et.al., 1967) that this pattern in which the spread and position of the response is similar in different species but the yield at optimum differs may have very little to do with nutrient response and could be due mainly to a selection for smaller plants. It is clear then that further analysis of the data is necessary.

Logarithmic transformation of the data allows the species to be compared in terms of their proportional

rather than their arithmetic differences. In this way, the nature of the response is more apparent, and if log yield is plotted against time the data yields information about growth rate. It was said that a greater response may be due to a greater growth rate as well as a greater or more efficient utilisation of nutrients. If this nutrient-response interaction is not changed by a logarithmic transformation it would suggest that a better uptake or use of nutrients is somehow involved in the difference observed between the species.

When an analysis of variance is performed on the logarithmic values of shoot dry weight of the first experiment, the response of the species appears to be significantly different. Moreover, the variance between nutrient levels is also significant. Further breakdown of the data shows that the species can be separated into two groups on the basis of their levels of significance. A. tenuis and F. rubra are not significantly different in their yields on a logarithmic basis, and L. perenne and D. glomerata are not significantly different. However, the A. tenuis and F. rubra species are each significantly different from both the L. perenne and D. glomerata species. A. tenuis is different from D. glomerata with a less than 5% probability that this difference is due to chance, and

from L. perenne with a 1% level of probability. F. rubra is different from L. perenne and D. glomerata with levels of probability of less than 5% and 10% respectively. Thus, the separation of the four species into two groups on the basis of response to variation in nutrient supply can be justified when response is measured in terms of shoot dry weight, at least under the conditions of these experiments.

Two other major experiments were conducted using these same four species. These were very similar and used the same basic nutrient solution, but in one only the kind and amount of nitrogen ion was varied. In the other, the amounts of NO_3-nitrogen and phosphorus were different from treatment to treatment. As these were designed primarily to discover biochemical differences between the species and not to analyse their growth patterns extensively, they will not be dealt with in this chapter, but will be described in later chapters.

CHAPTER 3

SOLUBLE SUGAR CONTENT AND NUTRIENT LEVEL

It has been seen that the previous chapter is not an isolated case of the demonstration of differences in the rates and patterns of growth among different plant species. In some cases, the relative growth rates of common species have been quite extensively studied (Grime and Hunt, 1975). Indeed, since Bradshaw and his co-workers (1964, IV) first suggested that interspecific differences in growth rate may be of great ecological importance, additional supporting evidence has accumulated from a number of sources (Driessche and Wareing 1966, Clarkson, 1967). If this apparent adaptation for reduced growth rate is a real effect, demonstrable in the field, and low growth rates could be important in ensuring the survival of plants on soils low in essential nutrients, then a mechanism must exist to control growth in these species.

There are a number of aspects of plant growth which can be examined to discover more about the nature of this apparent adaptation and which may shed light on any possible mechanism involved.

The first aspect to be examined is logically the carbohydrate accumulation of each of these species, especially under different nutrient conditions.

Researchers have repeatedly shown that plant growth is closely associated with the amount and even the character of carbohydrate and organic nitrogen content (Nightingale et.al., 1930; Hartwell et.al., 1913; Nightingale et.al., 1928; Jones et.al., 1965; Nowakowski and Cunningham, 1966; Watschke and Waddington, 1974; Waite, 1957; Ward and Blaser, 1961). Additionally, these factors vary tremendously with the degree of reproductive and vegetative vigour of the plant. The plant is after all composed largely of carbohydrates, carbohydrate derivatives and organic nitrogenous compounds. What remains is mostly water and a relatively low percentage of ash.

It is known that a plant may absorb large quantities of nitrate-nitrogen, but if this does not combine with the carbohydrate material to form proteinaceous compounds, little or no change in growth is noticable, unless of course the nitrate is present in such quantities as to be toxic to the plant (Nightingale, 1927). Carbohydrates and proteins are needed for the growth and/or the development of any of the structures of a plant and for the formation of new cells. Therefore, any change in the growth or development of a plant will cause related changes in its carbohydrate and organic nitrogenous constituents. It would be helpful then to know the amounts and kinds

of these compounds present in plant species in relation to nitrogen nutrition when comparing their growth. The soluble carbohydrates, that is, the non-structural or metabolic carbohydrates, of A. tenuis, L. perenne, D. glomerata and F. rubra will be examined in this chapter, and some nitrogenous compounds of these same species will be studied in the next.

Analysis of Soluble Carbohydrates

Pretreatment of Samples

Treatments of all species in each of the experiments were harvested as near the same time of day (early afternoon) as practically possible to minimise the effect of any diurnal differences in soluble carbohydrates. In the experiments in which only leaf and stem fractions were collected, the aerial parts of the plants in each pot were clipped even with the surface of the support material. Roots were also collected separately in one experiment. In this case, the grasses were supported on gauze discs over the liquid nutrient solutions, and the roots were clipped even with the lower surface of the gauze and rinsed in distilled water. Harvested material was killed in liquid nitrogen and freeze-dried. This procedure allowed a large number of samples to be harvested quickly and according to a strict schedule. These freeze-dried tissues were then stored in a freezer over calcium chloride ($CaCl_2$) in desiccators until extraction.

Extraction for Carbohydrate Analysis

The metabolic sugars were extracted from a known amount of sample using ethanol in a 100 °C water bath (Crawford and Huxter, 1977): The sugars were extracted

with 4 mls of 80% ethanol 3 times, the supernatent being decanted into a clean, labelled test tube each time. The solid residue was then extracted 3 times in 60% ethanol to remove the more insoluble polysaccharides. When amino acid analysis followed, 4 mls were removed before drying. In all cases, the total amount of extract was recorded.

The extracts from the experiments using different levels of Hoagland's solution were dried using forced air over a warm water bath. In later experiments, a vortex evaporator was used. These dried extracts were kept over phosphorus pentoxide (P_2O_5) in desiccators until required for the gas-liquid chromatography which followed.

Gas-Liquid Chromatography

The use of gas-liquid chromatography for both the qualitative and quantitative analysis of carbohydrates is now well established, and the method has been thoroughly researched (Bishop, 1962; Cayle et.al., 1968; Holligan, 1971; Holligan and Drew, 1971; Sweeley, 1963). As the use of gas-liquid chromatography is dependent on the volatility, among other things, of the substance being analysed, suitable derivatives of carbohydrates must be made and chromatographed. Trimethylsilyl (TMS) ethers of carbohydrates were used for all GLC analyses in this study. The work of Sweeley

(1963) showed that the TMS reaction of carbohydrates was virtually quantitative. Work since then has shown the reaction to be very nearly 100% (Holligan, 1971).

Although pyridine is the usual solvent used with carbohydrates, dimethylsulphoxide (DMSO) was used to redissolve the dried ethanolic extracts, as it has several advantages over pyridine in the preparation of TMS derivatives. The sugars dissolve more rapidly in DMSO. When DMSO is used, the reaction mixture separates into two phases, the upper one being identified as hexamethyldisiloxane. The TMS sugars have a high partition coefficient for this phase (Holligan, 1971), and it has been shown to contain virtually all the sugar derivatives (Ellis, 1969). This separation of the mixture into phases has an advantage for use with plant extracts, as interferring substances are removed to the lower phase.

The dried residue from the ethanol extractions was redissolved in a known amount of DMSO. The TMS derivatives were prepared by the following method. A 0.2 ml aliquot of redissolved extract was pipetted into a 'cherry' bottle reaction flask. The walls of the flask were then washed down with a further 0.2 ml of DMSO. A 0.2 ml aliquot of hexamethyldisilazan (HMDS) was then added, followed by 0.1 ml of trimethylchlorosilane (TMCS). The flask was then

quickly sealed with parafilm to keep out the moisture in the air. The flasks were then shaken in an automatic shaker for 2 minutes. These derivatives were then kept overnight in a phosphorus pentoxide desiccator.

When the two phases were formed and the derivatives ready to be used, additional DMSO was added to the lower phase with a long Pasteur pipette. This forced the upper phase into the graduated neck of the flask, and the volume of upper phase was measured accurately.

The TMS derivatives were analysed using a 5 foot (1.52m), coiled, glass column with an inside diameter of 4mm. This was filled with 1% methyl phenyl silicone gum (E.52) on a Diatomite C 'Q'(60-70 mesh) support. The column was filled and prepared according to P.J. Ridgeon (1969). This stationary phase is stable up to 290 °C.

1 ul aliquots were used in all samples. A standard temperature program was used in all cases for both standard sugars and samples. A temperature program of 2 minutes at 130 °C, followed by an increase of 6 °C per minute to 260 °C, followed by 15 minutes at 260 °C and a carrier gas flow-rate of 40 mls per minute, proved to separate the TMS carbohydrates sufficiently in the most convenient time. The chart recorder paper speed was 10mm/minute in all cases.

The TMS sugars were identified by preparing derivatives of authentic sugars in DMSO solution (Figure 3.1). These were run individually with samples of the four species used until most peaks were identified. The peaks remaining unidentified are labelled with numbers. When the peaks were identified as accurately as possible, 0.1% and 0.2% standard sugars solutions were made up with all the identified sugars dissolved in DMSO. These standards were run with samples in cases where identification was difficult, in order to ensure accuracy (Figure 3.2).

These same standards were used to relate peak area to its corresponding amount of sugar. The use of GLC in quantitive analysis depends on the linear relationship between the amount of a substance injected and the area of the resulting peak on the chromatogram. The usual method of peak area standardisation involves the injection of a known amount of a suitable standard, referred to as an internal standard, with the sample. The relationship between the amount of the internal standard and its peak area is used to establish the amount of each of the other components in the sample. This method is difficult to use with plant extracts, as quite often the peak of the internal standard interferes with the peaks of the components under study. Another disadvantage of using a single internal

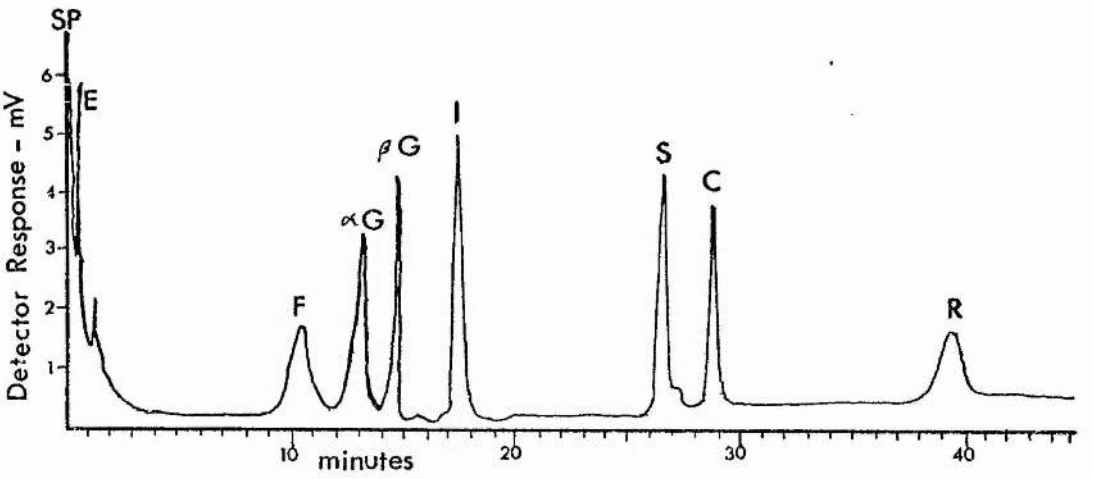


Figure 3.1: Gas-liquid chromatographic separation of a mixture of standard sugars and one sugar alcohol as their TMS ethers on an SE.52 column with a temperature program of 130-260 °C at 6 °C/minute with 2 minute and 15 minute periods at 130 °C and 260 °C respectively. The carrier gas (oxygen free nitogen) flow rate was 40 mls/minute, and the chart paper speed 10mm/min. SP, Solvent peak; E, Erythrose; F, Fructose; G, alpha Glucose; G, beta glucose; I, Inositol; S, Sucrose; C, Cellobiose; R, Raffinose. The area of the peak represents the detector response to the ether formed from 0.869µg of that particular sugar.

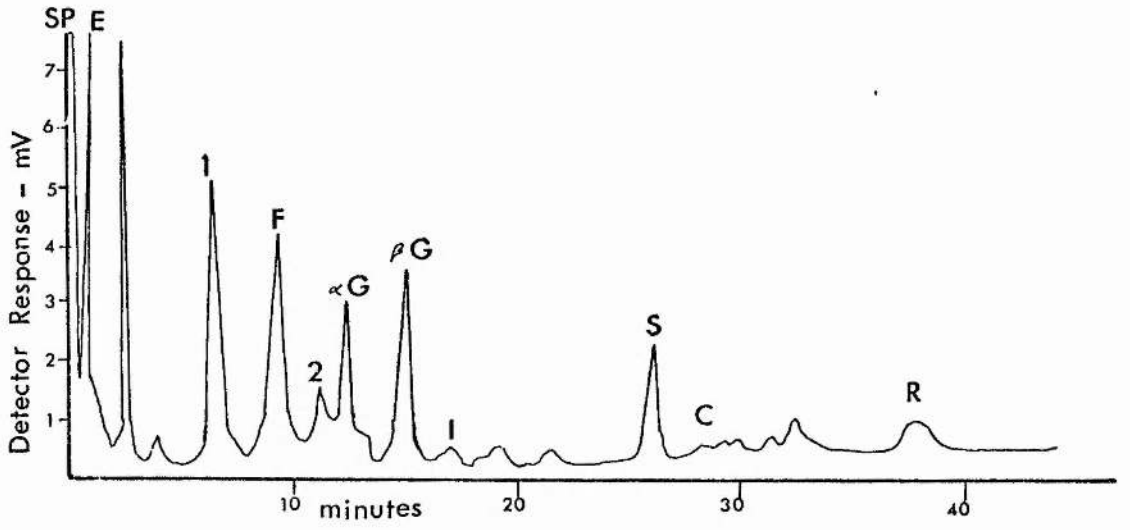


Figure 3.2: Gas-liquid chromatographic separation of soluble plant carbohydrates of Festuca rubra as their TMS ethers on an SE.52 column with a temperature program of 130-260 °C at 6 °C/min. with 2 minute and 15 minute periods at 130 °C and 260 °C respectively. The carrier gas (oxygen-free nitrogen) flow rate was 40 mls/min., and the chart speed was 10mm/min. SP, solvent peak; E, erythrose; 1, unidentified component; 2, unidentified component; G, alpha Glucose; H, Hexitols; G, beta Glucose; I, Inositol; S, Sucrose; C, Cellobiose; R, Raffinose. The remaining unidentified components were not measured.

standard is that the flame-ionisation detector (FID) of the GLC may not give the same response for different components. This may be related to molecular weight or other less tangible factors (Andrews, 1970). To avoid the introduction of this error, each identified peak in the sample was compared to its own sugar in the standard. When a peak was unidentified, a standard sugar very close to it was used. For example, the hexitol peak was calibrated with the glucose standard. Each time a group of samples was analysed, a group of standard derivatives was also run, each analysis with a different injection volume and therefore different weight of each standard sugar. This allowed linear regression of the amount of each standard sugar against its peak area (Figure 3.3). The actual weight of each TMS sugar in the samples could then be obtained from the regression line.

The peak area of each component was obtained by multiplying the height of a peak by its width at 1/2 the height. This result was then multiplied by the attenuation used for that peak. Although this method actually measures the triangle approximated by the peak, it has been shown that the precision of this method compares very favourably with that of other methods in common use (Mefford, 1968). When the chromatogram showed a multi-peak response for a TMS

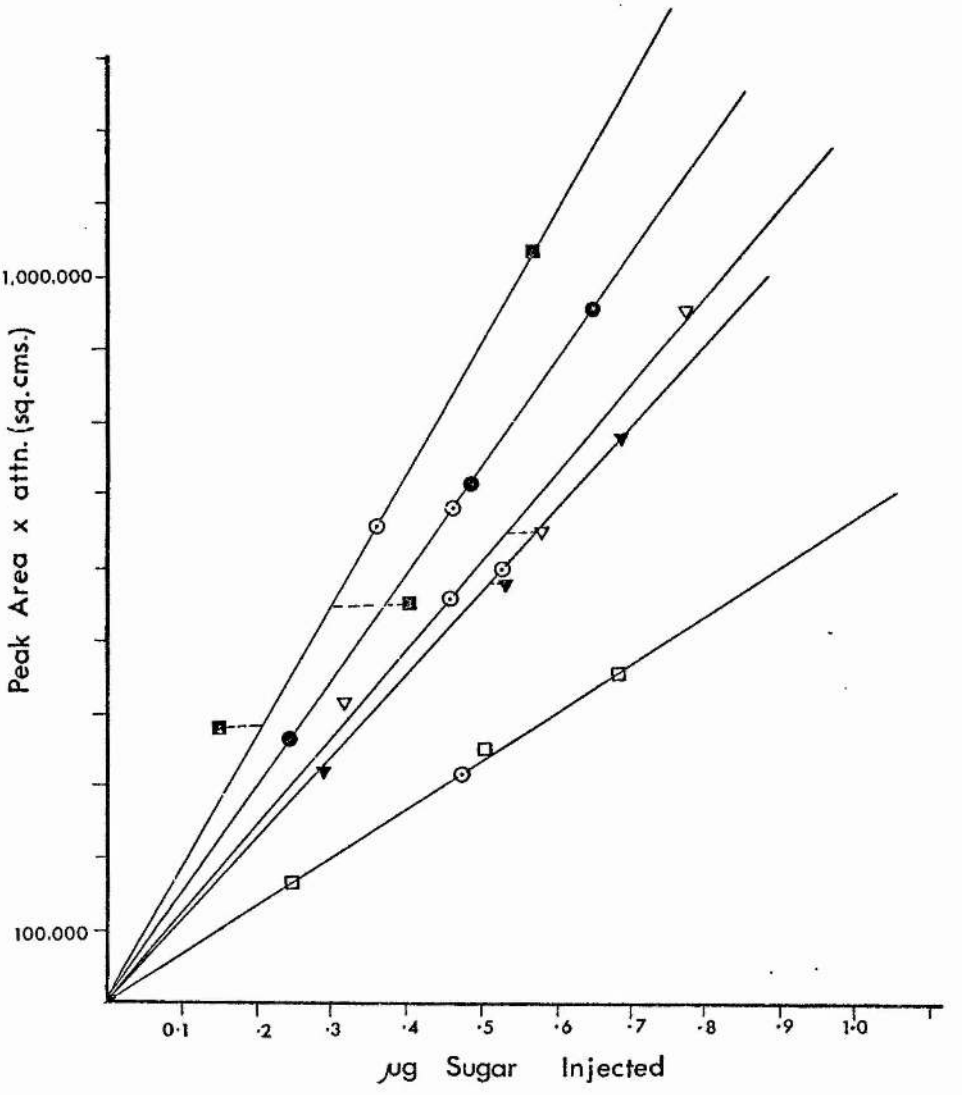


Figure 3.3: Example of one of the calibration curves for TMS Fructose ●, Glucose ■, Inositol ▽, Sucrose ▼, and Raffinose □ on a 1% SE.52 column. ⊙ indicates the mid-point of the regression line.

sugar due to the formation of alpha and beta anomers, as was the case for glucose, the areas of the peaks were added and treated as a single peak in the regression analysis.

In one experiment only, that measuring the response of 4 species to varying levels of phosphate and nitrate, the peak areas were measured electronically. This was possible, since the detector which the eluted components pass through gives an electrical signal proportional to the concentration of vapour of each component in the gas phase as it emerges from the column. Every 2 seconds an array of signals which measured the height of the peak (i.e. the detector response) at that particular moment was punched onto paper tape. The estimation of the area of a peak was achieved by the accumulation of the area under the curve based on these height measurements (Duncan, 1972). The data on the paper tape was read in by computer and processed by a program which smoothed the data, estimated the baseline, summed the data above the linear baseline, detected and located the number of peak complexes with 1-4 maxima, estimated the area of each component in any peak complex and printed the details of the analysis of the sample, recording the retention time of each component.

Effects of Different Concentrations of Complete Nutrient Solution on Soluble Carbohydrate Content

The first experiment in which the soluble carbohydrates were measured was described in detail in Chapter 2 (Page 11). In this experiment, seedlings of the 4 species under investigation, namely Lolium perenne, Dactylis glomerata, Agrostis tenuis and Festuca rubra, were grown individually in 4 treatments of increasing concentration of modified Hoagland's solution. There were 3 replicates of each species in each nutrient treatment. The 4 nutrient levels corresponded to 0.1%, 10%, 50% and 100% of this basic solution when made up to specifications. In terms of mM nitrogen, these levels were 0.016, 1.6, 9.0 and 16.0 respectively. After 6 weeks growth in the solutions, the seedlings were harvested, extracted in ethanol and dried.

The extracted residues were redissolved in DMSO, and TMS derivatives were prepared from them for gas-liquid chromatography. The TMS sugar peaks were recorded, peaks were identified using standard sugars run on the same day, and their amounts present in the tissues were determined by comparison of the peak areas with peak-area calibration curves. The principal sugars identified were the following: sucrose, fructose, glucose and raffinose. Inositol and hexitol peaks were

also identified.

Results

In this preliminary experiment, the 3 replicates each consisted of individual seedlings. In order to make up a sufficient weight for analysis, the three replicates were combined and extracted together. The carbohydrate analysis was then, in effect, an investigation of the effect of 2 experimental factors (e.g. generic differences and nutrient level differences) with no replication. The results for this experiment are presented in Table 3.1.

Table 3.1a-e: Soluble carbohydrate content of 4 species after 6 weeks growth in each of 4 levels of modified Hoagland's solution. The three replicates of each species in each treatment in this experiment were combined into one sample for analysis. Data are expressed as mg sugar per g dry weight.

Table 3.1a: Fructose content

%conc. Hoagland's solution	D.glomerata	L.perenne	A.tenuis	F.rubra
0.1	0.1357	0.1611	0.0	0.208
10	0.1415	0.1047	0.0	0.1164
50	0.0656	0.1099	0.0649	0.3523
100	0.0444	0.0196	0.0551	0.0356

Table 3.1b: Sucrose content

%conc. Hoagland's solution	D.glomerata	L.perenne	A.tenuis	F.rubra
0.1	2.5997	5.7800	1.8688	5.6602
10	0.6019	0.5896	0.3891	0.7702
50	0.5874	2.6548	3.5256	4.7624
100	0.3565	0.2627	3.1771	3.5319

Table 3.1c: Inositol content

%conc. Hoagland's solution	D.glomerata	L.perenne	A.tenuis	F.rubra
0.1	0.0609	0.1329	0.0113	0.1086
10	0.0259	0.0381	0.0210	0.0193
50	0.0181	0.0474	0.0180	0.0818
100	0.0207	0.0052	0.0302	0.0622

Table 3.1d: Hexitol content

%conc. Hoagland's solution	D.glomerata	L.perenne	A.tenuis	F.rubra
0.1	0.4989	0.6890	0.1107	0.9490
10	0.4166	0.8137	0.1243	2.4241
50	0.1867	0.2899	0.2813	1.3535
100	0.1170	0.0391	0.1806	0.9482

Table 3.1e: Glucose content

%conc. Hoagland's solution	D.glomerata	L.perenne	A.tenuis	F.rubra
0.1	0.2246	0.5179	0.0871	0.7556
10	0.0283	0.0029	0.1097	0.0712
50	0.1718	0.0484	0.2389	0.4915
100	0.0867	0.0050	1.1657	0.1031

The data presented in these tables reveal a few patterns. The species from eutrophic environments, D. glomerata and L. perenne, tend to show a decrease in amount of these sugars and sugar alcohols per gram dry weight with increasing concentration of nutrients.

These species also show high levels of these carbohydrates at very low levels of nutrition. The species from relatively oligotrophic environments, A. tenuis and F. rubra, while showing the same accumulation of these carbohydrates at very low nutrient levels followed by a sharp decrease at the 10% nutrient level, differ in that their carbohydrate content increases when these species are grown in solutions of high nutrient concentration. Thus, when grown in a range of levels of complete nutrient solution, species adapted to oligotrophic and eutrophic environments may be distinguished by their different patterns of sugar accumulation over this range of levels. Although these patterns were not dramatic, they were clear and warranted further investigation.

Effects of Solutions of Different Nitrogen Concentration on Soluble Carbohydrate Content

Having finished the experiment involving different levels of modified Hoagland's solution, the next step was to vary only the kinds and quantity of single nutrient ions to see if a particular element or elements could account for the observed effects. It was decided to vary only the nitrogen content of the solutions first. Nitrogen was the logical choice for several reasons. It is now accepted that nitrogen is one of the most important soil nutrients, and it is one of the most generally deficient nutrient factors limiting the growth of crop species in natural soils (Black, 1957). Further, nitrogen deficiency is known to dominate the effects of the other elements and can have overriding control of growth (Hewitt and Smith, 1974).

Differences in nitrogen level have been shown to have important effects on growth in the field as well as in the laboratory. It is known that different natural habitats can have very different nitrogen concentrations in their soils (Olsen, 1921; Millar, 1955). Even very small differences in nitrogen concentration can have a marked effect on botanical composition if other factors, such as competition, are in operation. Finally, there is evidence that different species can differ in their response to nitrogen (Bradshaw et.al., 1964).

In order to test the effects of different levels of nitrogen, it was necessary to use a nutrient solution other than the Hoagland's solution used in the previous experiments. That solution contained nitrogen in the form of KNO_3, $Ca(NO_3) \cdot 4H_2O$, and $NH_4H_2PO_4$. If this solution were used in the nitrogen experiments, it would be impossible to separate the effects of NO_3-N and NH_4-N. Therefore, solutions were developed in which the nitrogen, either in the form of ammonium or nitrate ions, could be varied in concentration. A modified version of the nutrient solutions employed by E.A. Kirkby (1969) was used. Kirkby used the following solutions (me./l).

NO_3-solution	
$Ca(NO_3)$	5.0
KH_2PO_4	2.0
$MgSO_4$	1.5

NH_4-solution	
$(NH_4)_2SO_4$	5.0
KH_2PO_4	2.0
$MgSO_4$	1.5
$CaSO_4$	5.0

If the above solutions are changed so that different nitrate levels are used, Ca++ ions also vary. To allow for this, the amount of $CaSO_4$ in each treatment was adjusted so that calcium ion concentration remained the same. This meant that sulphate was the only variable anion other than nitrate. This additional difference between the treatments was acceptable since it is very probable that sulphate is the ion least likely to affect or be

affected by the uptake of other ions (Kirkby, 1969). The micronutrients added were the same as in the Hoagland's solution and in the same amounts. A comparison of the nutrient solutions used in this experiment with the Hoagland's solution follows (me./l).

Hoagland's solution		NO_3-solution		NH_4-solution	
KNO_3	6.0	KH_2PO_4	2.0	KH_2PO_4	2.0
$Ca(NO_3)_2.4H_2O$	4.0	$Ca(NO_3)_2.4H_2O$	5.0	$(NH_4)_2SO_4$	5.0
$NH_4H_2PO_4$	2.0	$MgSO_4.7H_2O$	1.5	$MgSO_4.7H_2O$	1.5
$MgSO_4.7H_2O$	1.0	$CaSO_4.2H_2O$	*	$CaSO_4.2H_2O$	5.0

* Variable

There were three basic parts to the experiment. In one, nitrate was the only form of nitrogen in the treatments. There were 6 treatments, 2 replicates each, and the levels of nitrate in me./l were 4, 8, 16, 24, 32 and 48. Secondly, there were 6 treatments in which ammonium was the only nitrogen source. These also had 2 replicates each, and the NH_4 ions were present in the same me./l concentrations as the NO_3 ions above. The third part of the experiment used a single level of nitrogen-containing ions (i.e. both NH_4 and NO_3 ions), but the proportions of the two ions were different in each treatment. The me./l of each ion, NO_3 and NH_4 were

24 and 0, 20 and 4, 16 and 8, 12 and 12, 8 and 16, 4 and 20, and 0 and 24. Again there were two replicates per species per treatment, and all the other macronutrients were at a constant level. In addition to the macronutrients, each treatment also contained 0.9 me./l Fe^{+++} as Fe-EDTA and micronutrients in the following amounts (me./l): 0.05 Cl^-, 0.075 B^{+++}, 0.004 Mn^{++}, 0.004 Zn^{++}, 0.001 Cu^{++} and 0.002 Mo^{++++}.

At the start of this experiment, the pH of each of the solutions was adjusted to 5.5. During the course of the experiment, the pH of each solution was checked twice weekly and adjusted at these times to 5.5 with dilute $Ca(OH)_2$ or 0.02N H_2SO_4 solutions. Every two weeks new nutrient solutions were made up, and the old solutions were completely replaced.

As in the second complete nutrient solution experiment, the same uniform numbers of seeds per species were placed on non-absorbent cotton wool in identical pots. These pots were then kept watered with distilled water until germination, when the pots were each placed in the appropriate nutrient solution. The pots were set out in an unheated glasshouse in random arrangement, and rotated twice weekly. The four species were grown in the different nutrient solutions for 6 weeks during July and August 1975. At harvest, only the shoots were taken. These were killed in liquid nitrogen

and freeze-dried. The dried samples were stored in vials and kept in a freezer at -20°C in desiccators over calcium chloride until analysis. The dry weights were measured at the time the dry material was removed for chemical analysis according to the above method.

The Results

In this experiment, although all sugars were measured, only the reducing sugars glucose and fructose and the non-reducing sugar sucrose will be examined in detail. The three parts of this experiment will be examined separately. The effects of NO_3-N on carbohydrate content will be discussed first, followed by discussions of the effects of NH_4-N and the effects of NO_3-N/NH_4-N combination treatments.

Tables 3.2a-c show the average amounts of fructose, glucose and sucrose in these species. The trends shown in these tables are more clearly illustrated in Figures 3.4a-c. These show how the content of each sugar is affected by nitrate concentration in the four species. The Figures 3.5a-d show the amounts of these sugars in each species separately.

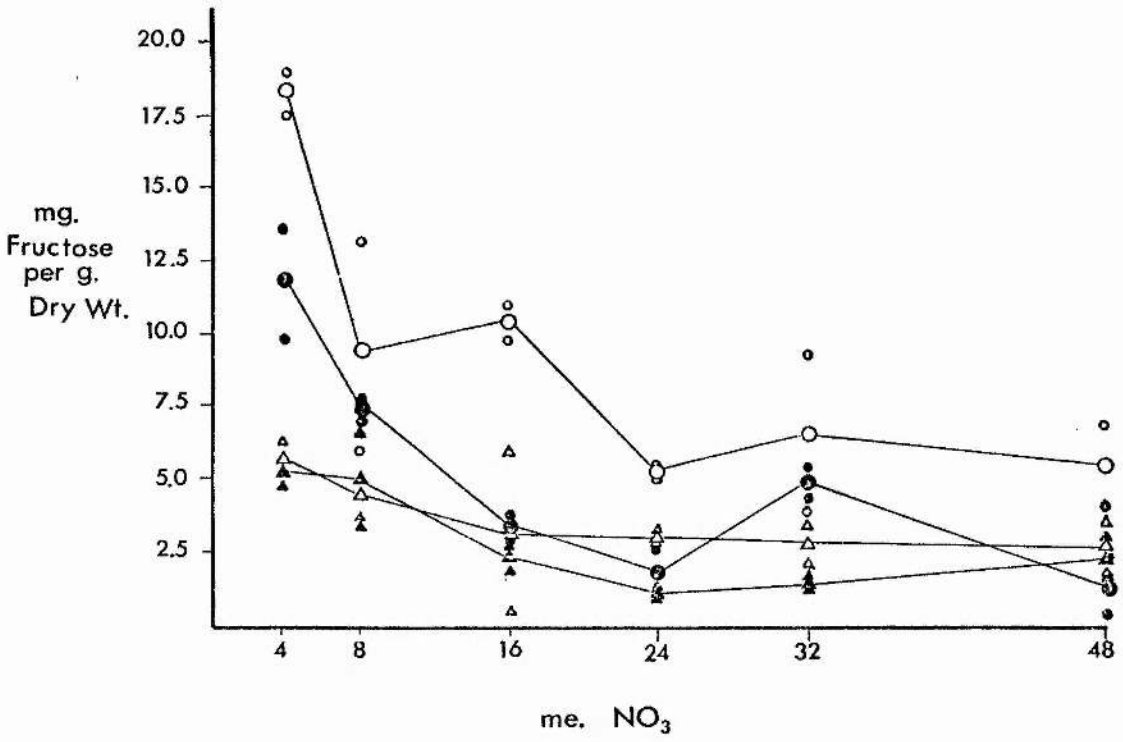


Figure 3.4a: Effect of 4,8,16,24,32 and 48 me./l NO_3 on the fructose content of L.perenne, A.tenuis, D.glomerata and F.rubra. The amount of sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the 2 replicates are connected by lines. The actual values of the replicates are also shown.

○= L.perenne ●= D.glomerata
▲= A.tenuis △= F.rubra

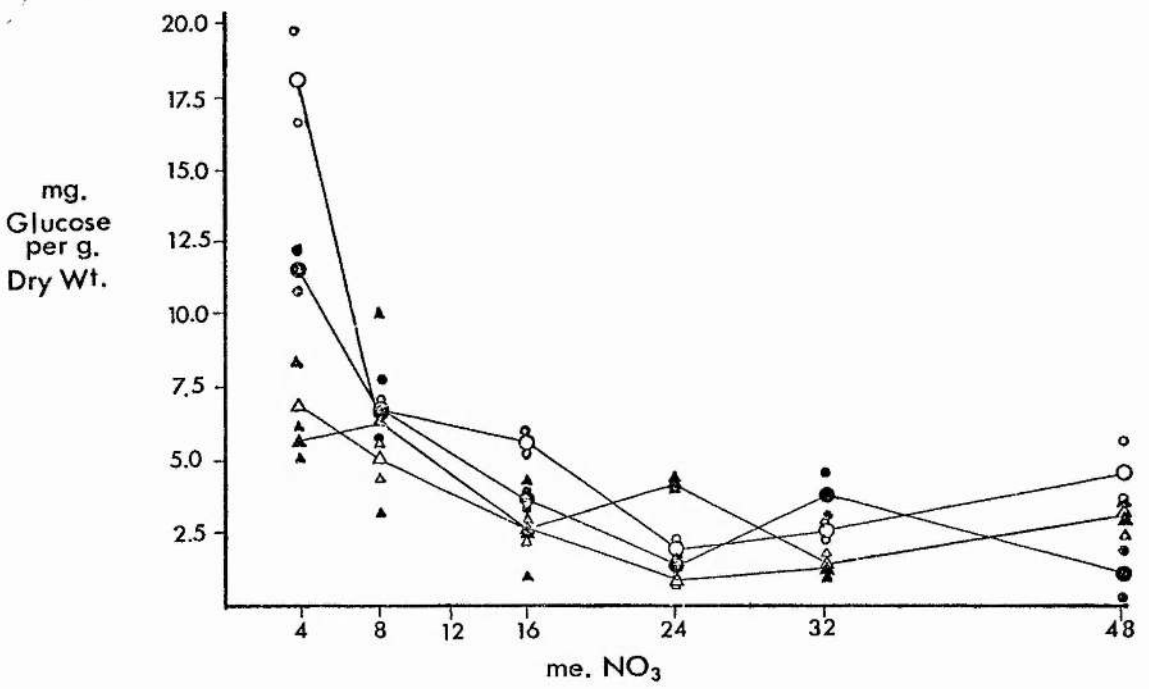


Figure 3.4b: Effect of 4,8,16,24,32 and 48 me./l NO_3 on the glucose content of 4 species. The amount of sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the 2 replicates are connected by lines. The actual values of the replicates are shown.

○= L.perenne ●= D.glomerata
▲= A.tenuis △= F.rubra

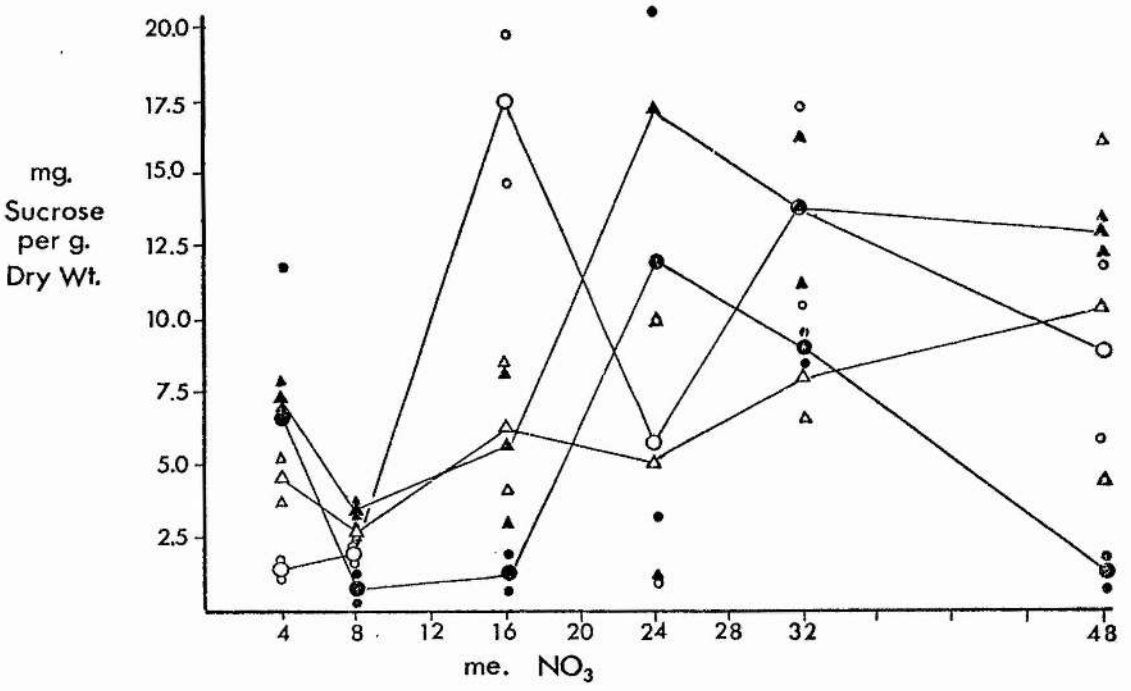


Figure 3.4c: Effect of 4,8,16,24,32 and 48 me./l NO_3 on the sucrose content of 4 species. The amount of sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines. The actual values of the replicates are shown.

○= L.perenne ●= D.glomerata
▲= A.tenuis △= F.rubra

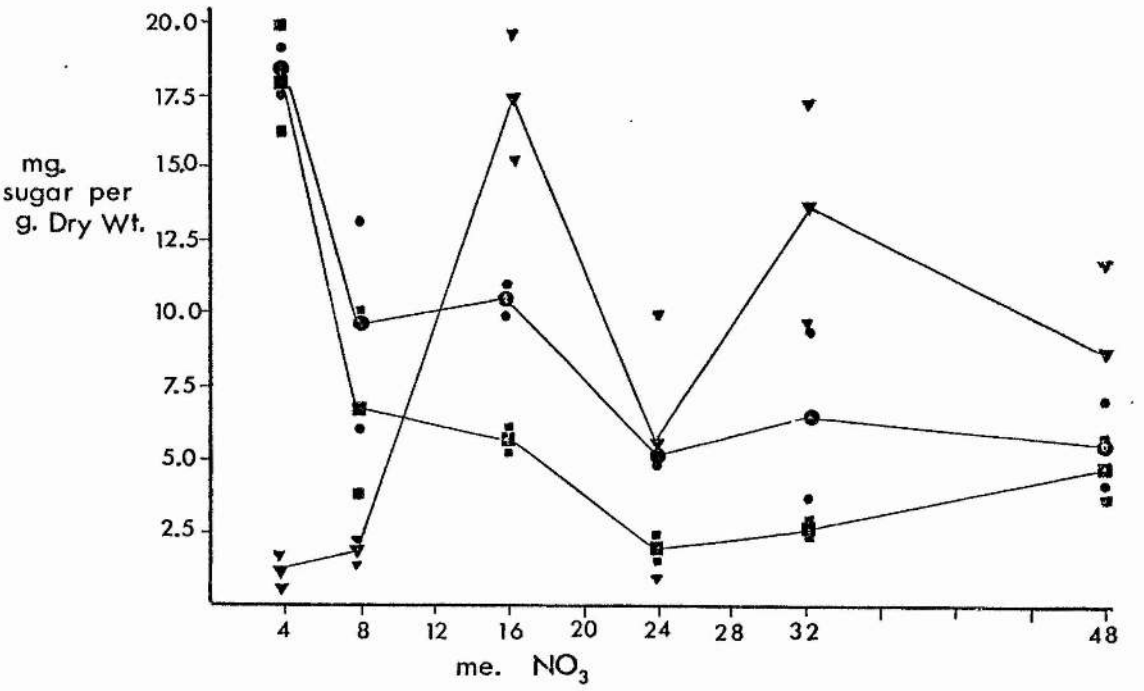


Figure 3.5a: The content of 3 sugars in L.perenne shoots grown for 6 weeks in 6 treatments of different NO_3 concentration. Each result is the mean of 2 replicates and is presented in terms of mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

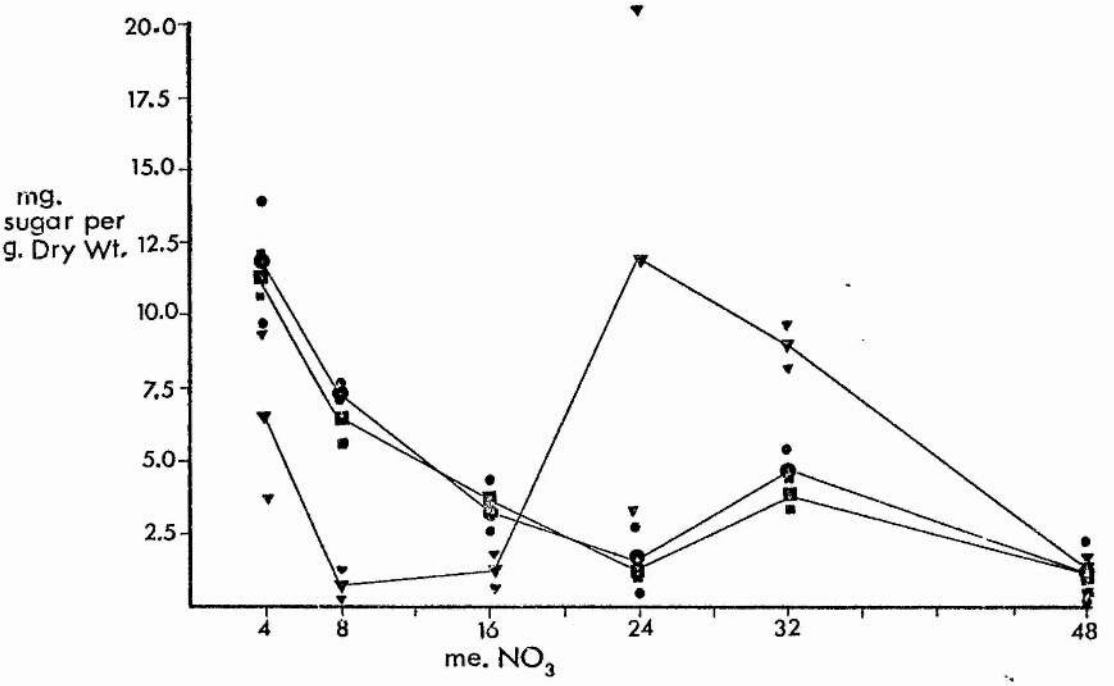


Figure 3.5b: The content of 3 sugars in D.glomerata shoots grown for 6 weeks in treatments of different NO_3 concentration. Each result is the mean of 2 replicates and is presented in terms of mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

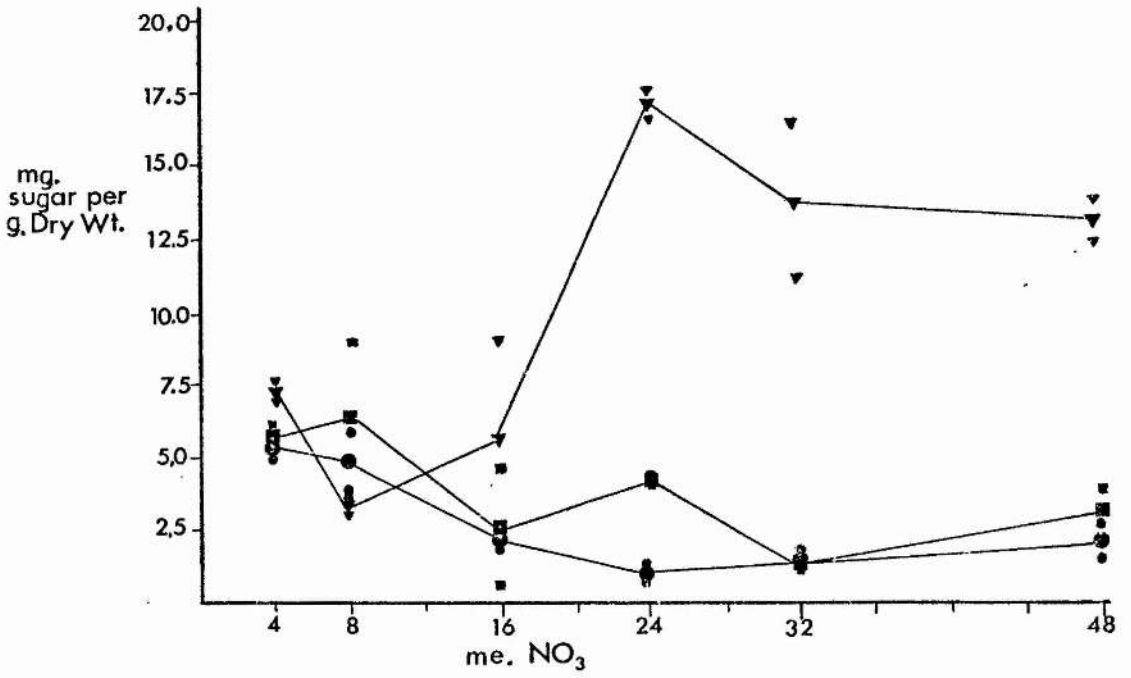


Figure 3.5c: The content of 3 sugars in A. tenuis shoots grown for 6 weeks in treatments of different NO_3 concentration. Each result is the mean of 2 replicates and is expressed as mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

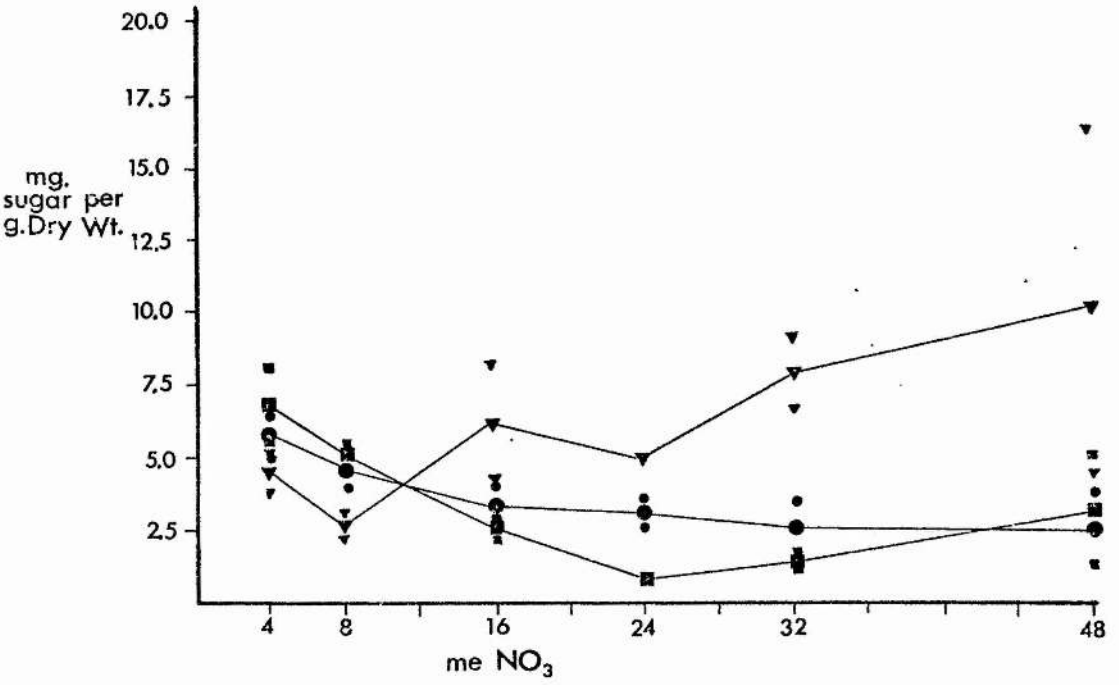


Figure 3.5d: The content of 3 sugars in F.rubra shoots grown for 6 weeks in 6 treatments of different NO_3 concentration. Each result is the mean of 2 replicates and is expressed as mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

Table 3.2a-c: Influence of 4, 8, 16, 24, 32 and 48 me./l NO_3-N on soluble carbohydrate content of 4 species. Two replicates of each species were grown for 6 weeks in treatments of a balanced nutrient solution in which only the concentration of NO_3-N varied. Results are expressed as the mean of the two replicates (each of which was analysed separately) in terms of mg sugar per gram shoot dry weight.

Table 3.2a: Fructose content

me./l NO_3-N	D.glomerata	L.perenne	A.tenuis	F.rubra
4	12.0071	18.4592	5.4329	5.7379
8	7.6231	9.4850	4.7935	4.4688
16	3.7644	10.6873	3.6978	2.4746
24	2.2294	5.2571	3.0621	1.0000
32	4.8081	6.6411	1.7692	1.3713
48	1.5116	5.5985	2.8532	2.4488

Table 3.2b: Glucose content

me./l NO_3-N	D.glomerata	L.perenne	A.tenuis	F.rubra
4	11.4723	16.6318	5.7215	6.9269
8	6.9004	7.0148	6.5232	4.9607
16	3.2687	5.7332	2.8609	2.6038
24	1.6653	1.8447	4.3399	1.0000
32	3.9098	2.4785	1.5447	2.0541
48	1.2572	4.3711	3.3559	3.1006

Table 3.2c: Sucrose content

me./l NO_3-N	D.glomerata	L.perenne	A.tenuis	F.rubra
4	6.7576	1.3419	7.3237	4.5248
8	0.6334	1.8437	3.4319	2.2033
16	1.0886	17.4897	5.9479	5.5155
24	12.0745	5.47902	17.0454	5.0000
32	8.9294	13.7265	13.8952	7.6486
48	1.1570	8.7336	12.7611	10.2699

Again, as with the preliminary experiment, some patterns are quite obvious. With the amount of NO_3-N as the only difference between treatments, the reducing sugars of all the species investigated show a very similar pattern of gradual decrease of sugar level with increasing me. of NO_3-N. In this experiment, there was no obvious difference between the species of oligotrophic and those of eutrophic environments when the reducing sugars are considered. The patterns of the non-reducing sugar sucrose, however, are not so similar in the four species. A. tenuis and F. rubra maintain a higher sucrose level at higher concentrations of NO_3-N than either L. perenne or D. glomerata. If these species differ in sugar concentration as the preliminary experiment suggested, the effect, at least with the reducing sugars, is probably not due solely to difference in nitrate.

When the effects of nitrate-nitrogen were analysed separately from the other treatments, analysis of variance showed that the variability due to the treatment effects was very significant for all three sugars: $p<0.001$ for fructose and glucose, and $p<0.01$ for sucrose. The variability due to species differences was highly significant for fructose ($p<0.001$), very significant for glucose ($p<0.01$) and just significant

($p<0.05$) for sucrose. When the least significant differences ($p<0.05$) between the means of the 6 treatments of each species were considered, there was no significant difference between *A. tenuis* and *F. rubra* in either fructose or glucose. *L. perenne* and *D. glomerata* were not significantly different in their responses of glucose to NO_3-N. The response of sucrose to nitrate in these species was quite different. Analysis showed no significant difference between *A. tenuis* and *L. perenne*, while *F. rubra* and *D. glomerata* were not significantly different.

It seems, therefore, that although these species do not differ in the pattern of their response to soluble carbohydrate concentration to nitrate level, they do differ in their degree of response. Further, the responses of *F. rubra* and *A. tenuis* are very similar in the reducing sugars, as are *L. perenne* and *D. glomerata*.

The second part of this experiment used a complete nutrient solution in which only the amount of NH_4-N was varied in six treatments. There was no NO_3-N present in the solutions. These results are presented in the following tables.

Tables 3.3a-c: Influence of 4, 8, 16, 24, 32 and 48 me./l NH_4-N on soluble carbohydrate content of 4 species. Two replicates of each species were grown for 6 weeks in treatments of a balanced nutrient solution in which only the concentration of NH_4-N varied. Results are expressed as the average of the 2 replicates (each of which was analysed separately) in terms of mg. sugar per gram shoot dry weight.

Table 3.3a: Fructose content

me./l NH_4-N	D.glomerata	L.perenne	A.tenuis	F.rubra
4	7.0267	20.7956	5.8101	7.4001
8	7.8347	6.1094	13.5302	14.5008
16	13.8575	35.9218	13.3499	14.4823
24	14.5561	24.2578	11.5828	13.5126
32	4.3524	4.6609	9.9225	8.6524
48	4.0928	8.166	1.9699	1.7117

Table 3.3b: Glucose content

me./l NH_4-N	D.glomerata	L.perenne	A.tenuis	F.rubra
4	7.6330	13.3925	6.1373	10.5957
8	9.2333	5.7990	24.5383	19.2603
16	14.1629	31.6107	18.3756	27.8468
24	15.4186	20.2081	16.5174	20.4729
32	5.3594	3.4971	14.1596	16.4807
48	8.0659	7.7621	15.0363	6.3076

Table 3.3c: Sucrose content

me./l NH_4-N	D.glomerata	L.perenne	A.tenuis	F.rubra
4	1.6133	10.9605	9.5029	1.9438
8	0.1654	1.7671	4.4106	2.2646
16	3.5189	19.7164	4.4724	9.0825
24	2.2375	6.4951	2.1015	5.3938
32	8.5149	12.3981	6.9725	22.8064
48	25.5967	24.3409	23.0135	14.0533

As before, these data are also presented in graphical form and are illustrated in Figures 3.6 and 3.7. These figures show the content of the individual sugars in all four species, and the content of the three sugars in the individual species, respectively. The effects of NH_4-N on soluble carbohydrate content appears to be quite different from those of NO_3-N. With varying NH_4-N, L. perenne showed a rapid decrease in reducing sugars from the 4 to the 8 me./l NH_4 level (for fructose this was marked, and this species showed values much lower than those reached by the other species). This was followed by an accumulation of reducing sugars, which brought the concentration of each sugar to between 30 and 38 mg sugar per gram dry weight. L. perenne also showed a decrease at the 32 me./l level followed by an increase in sugar concentration approaching the 48 me./l NH_4 level. The pattern of response of D. glomerata was similar, but there was apparently a continuous increase in reducing sugar concentration until the 24 me./l NH_4 level was reached. Further, the amount of sugar accumulated in the shoots of this species in the 16 and 24 me./l NH_4-N levels was only about 1/2 of that seen in L. perenne. This was followed by a decrease in sugar concentration around 32 me./l NH_4. Again, as in the Lolium species, sugar concentration increased as the highest (48 me./l)

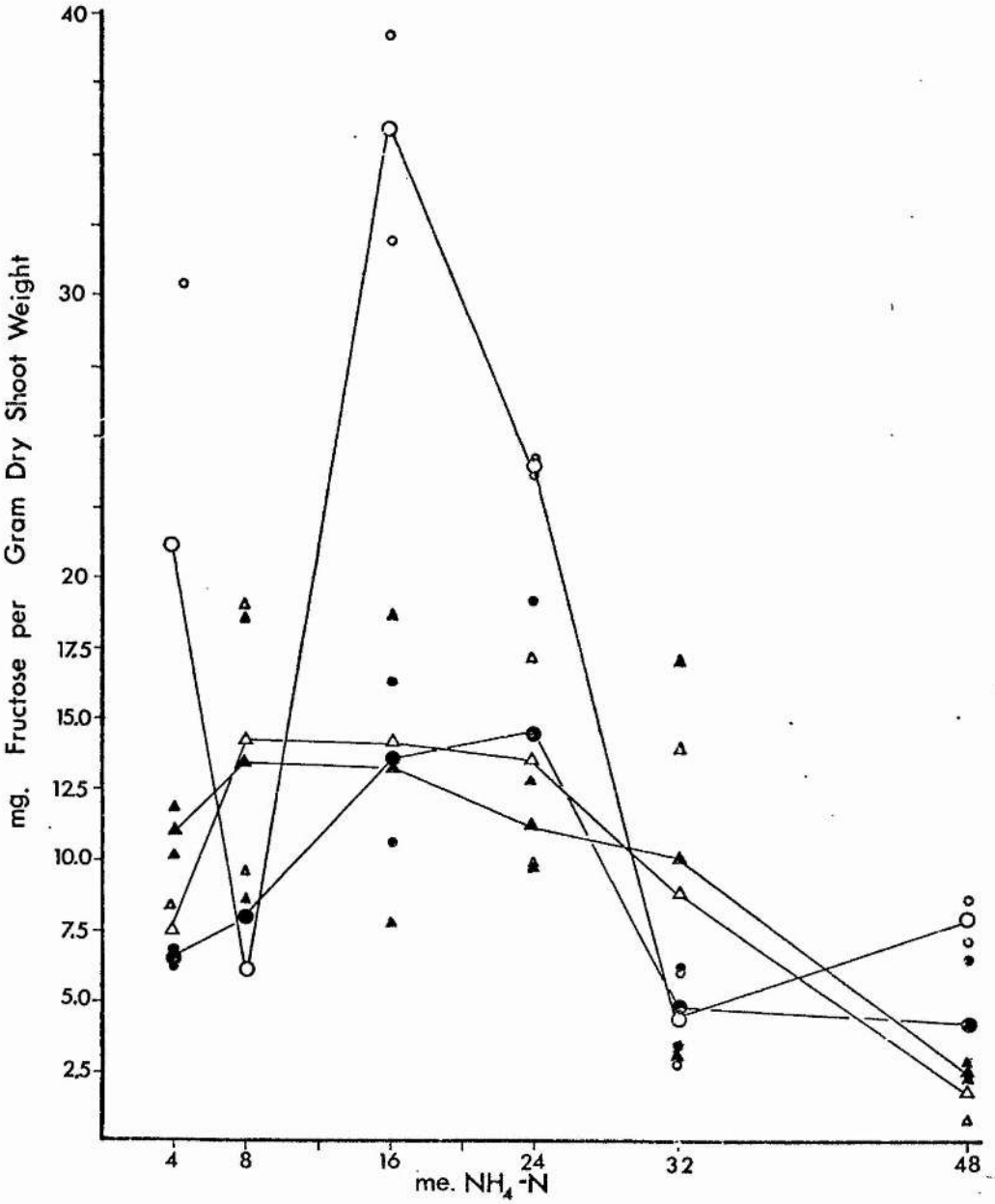


Figure 3.6a: Effect of 4,8,16,24,32 and 48 me./l NH_4 on the fructose content of 4 species. The amount of this sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines. The actual values of the replicates are shown.

○= L. perenne ●= D. glomerata
▲= A. tenuis △= F. rubra

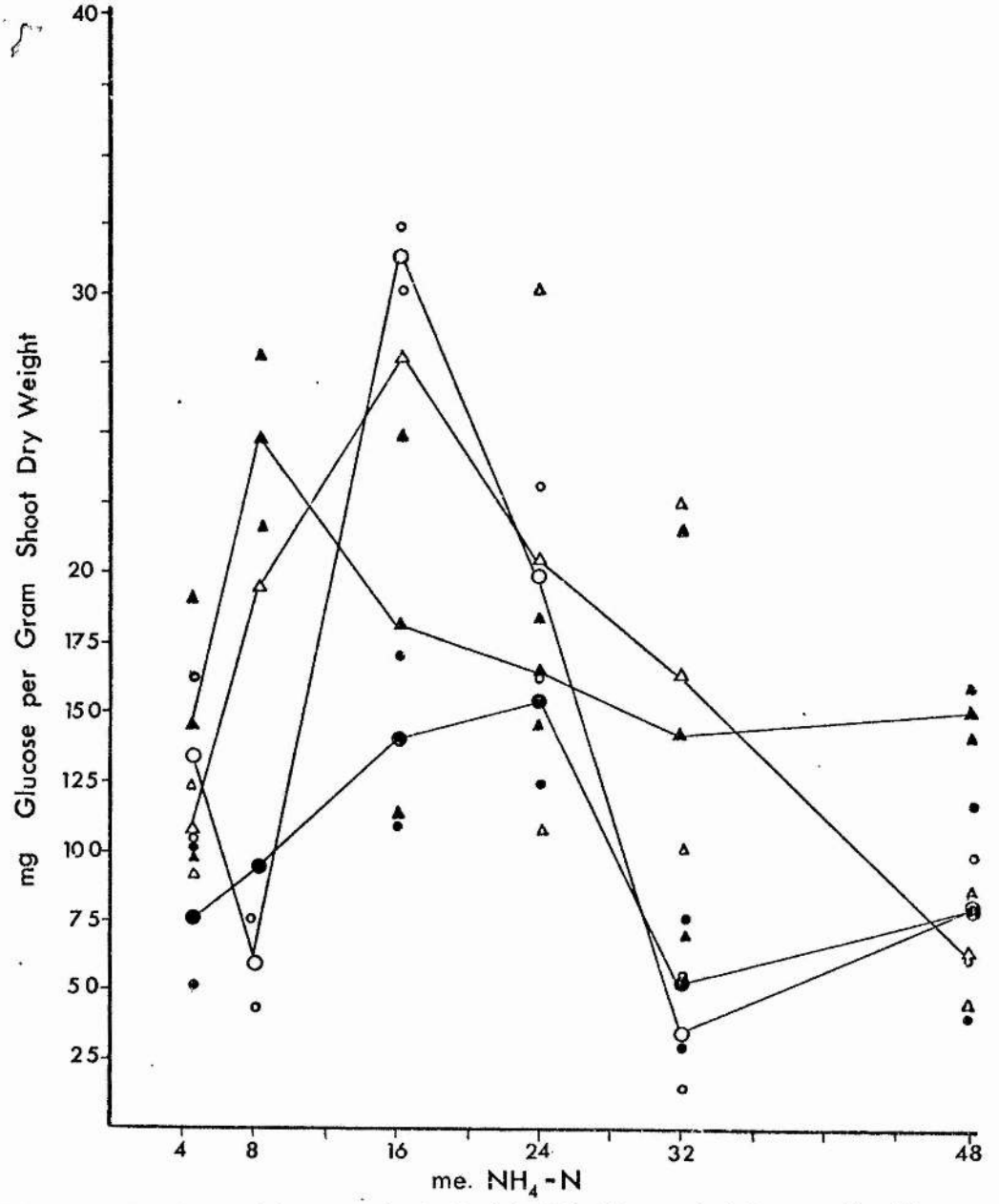


Figure 3.6b: Effect of 4,8,16,24,32 and 48 me./l NH_4 on the glucose content of 4 species. The amount of this sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines. The actual values of the replicates are shown.

○ = L.perenne ● = D.glomerata
▲ = A.tenuis △ = F.rubra

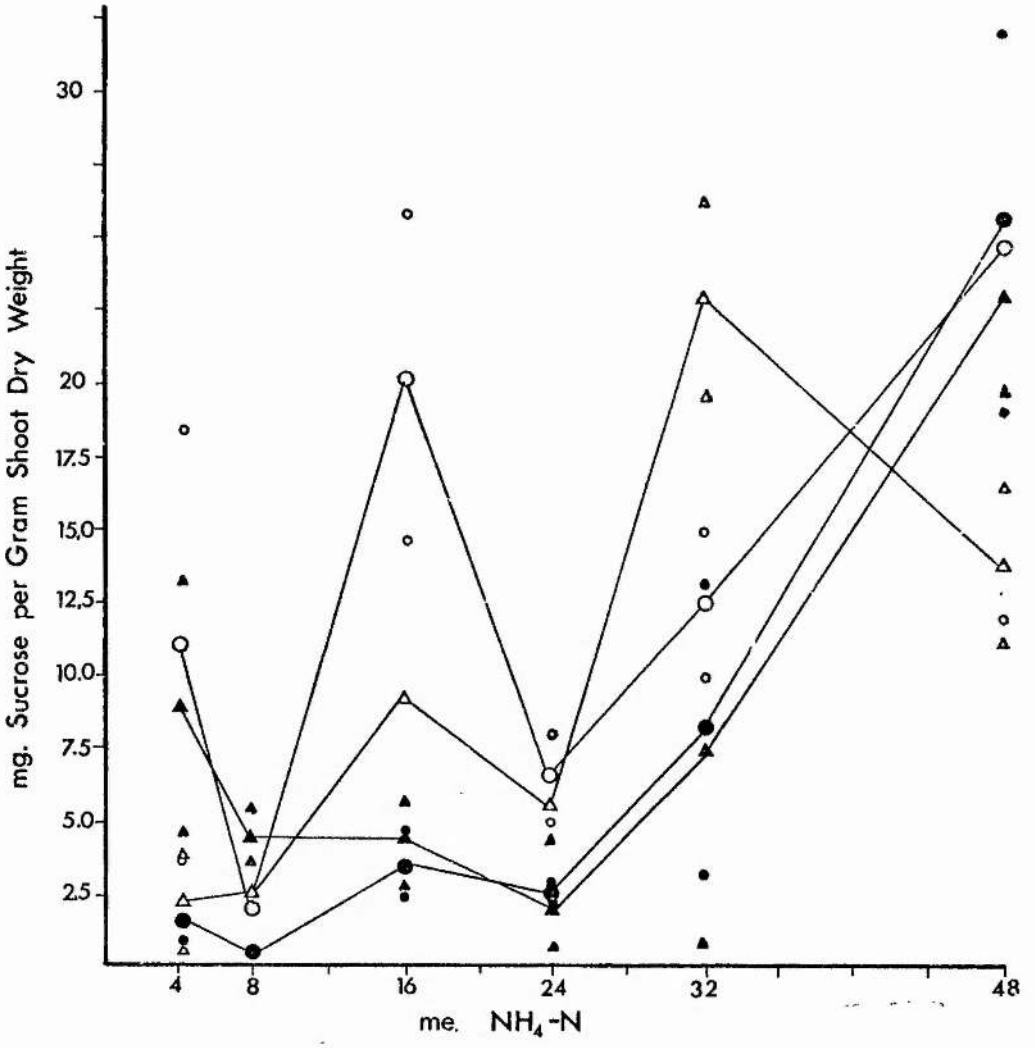


Figure 3.6c: Effect of 4,8,16,24,32 and 48 me./l NH_4 on the sucrose content of 4 species. The amount of this sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines. The actual values of the replicates are shown.

○= L.perenne ●= D.glomerata
▲= A.tenuis △= F.rubra

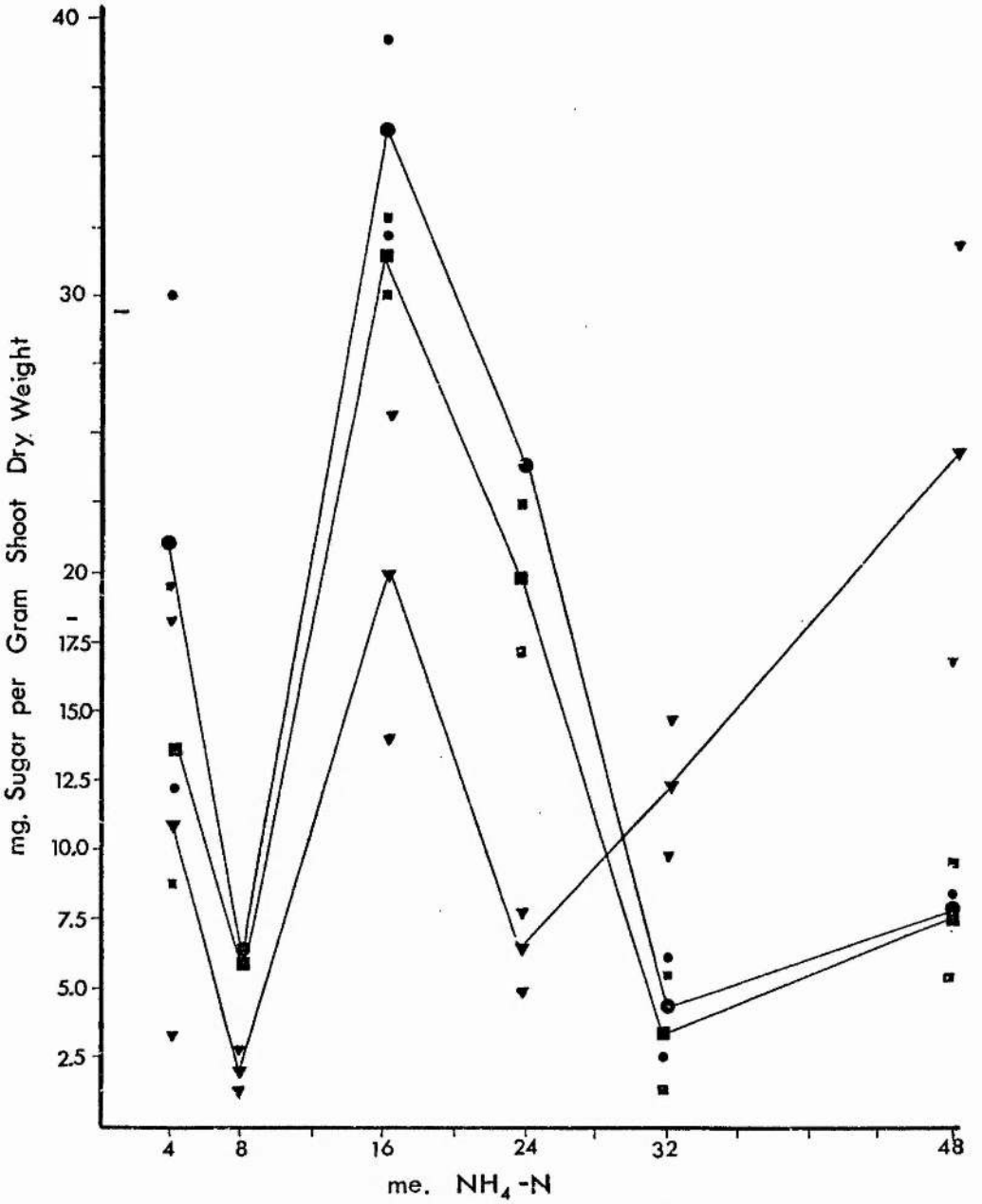


Figure 3.7a: The content for 3 sugars in L.perenne shoots grown for 6 weeks in 6 treatments of different NH_4 concentrations. Each result is the mean of 2 replicates and are presented in terms of mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

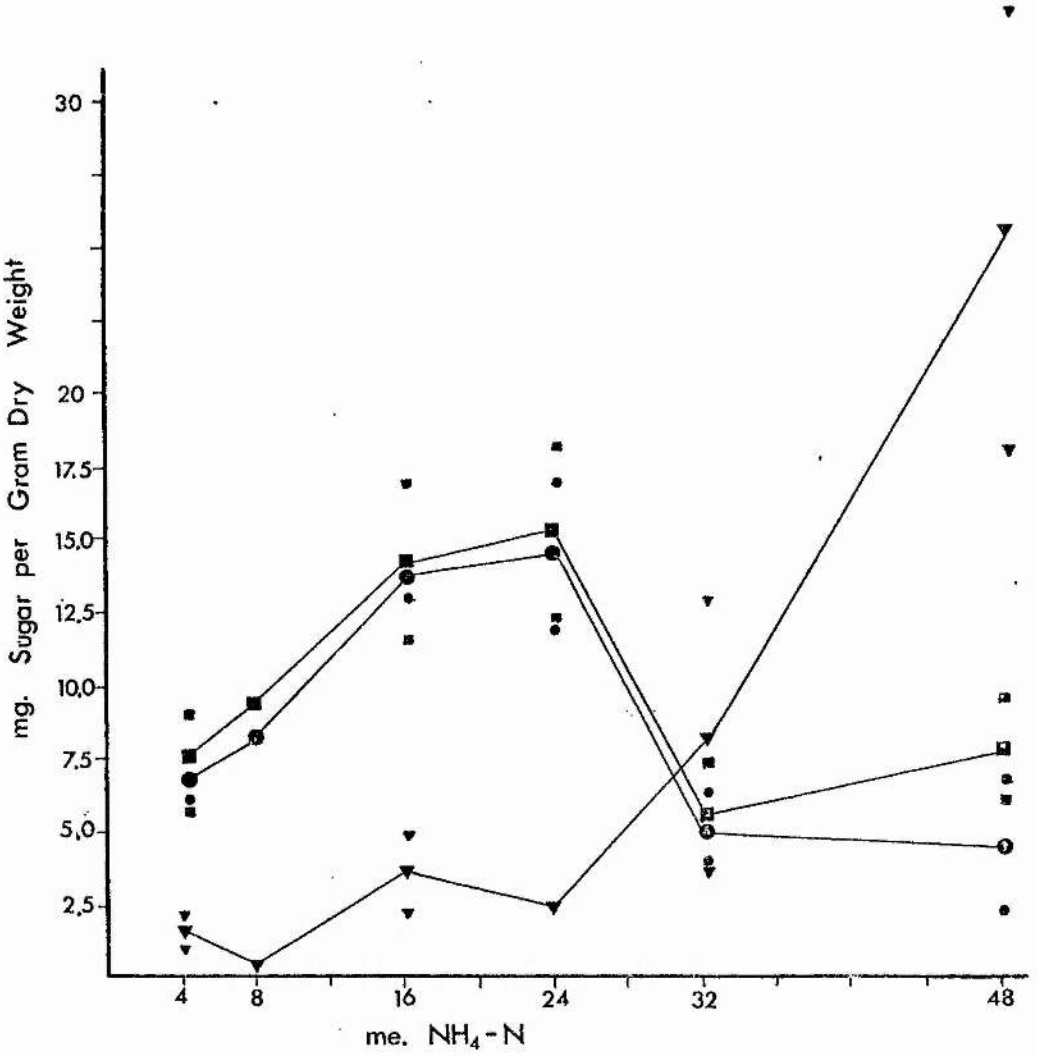


Figure 3.7b: The content of 3 sugars in D.glomerata shoots grown for 6 weeks in treatments of different NH_4 concentration. Each result is the mean of 2 replicates and is presented in terms of mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

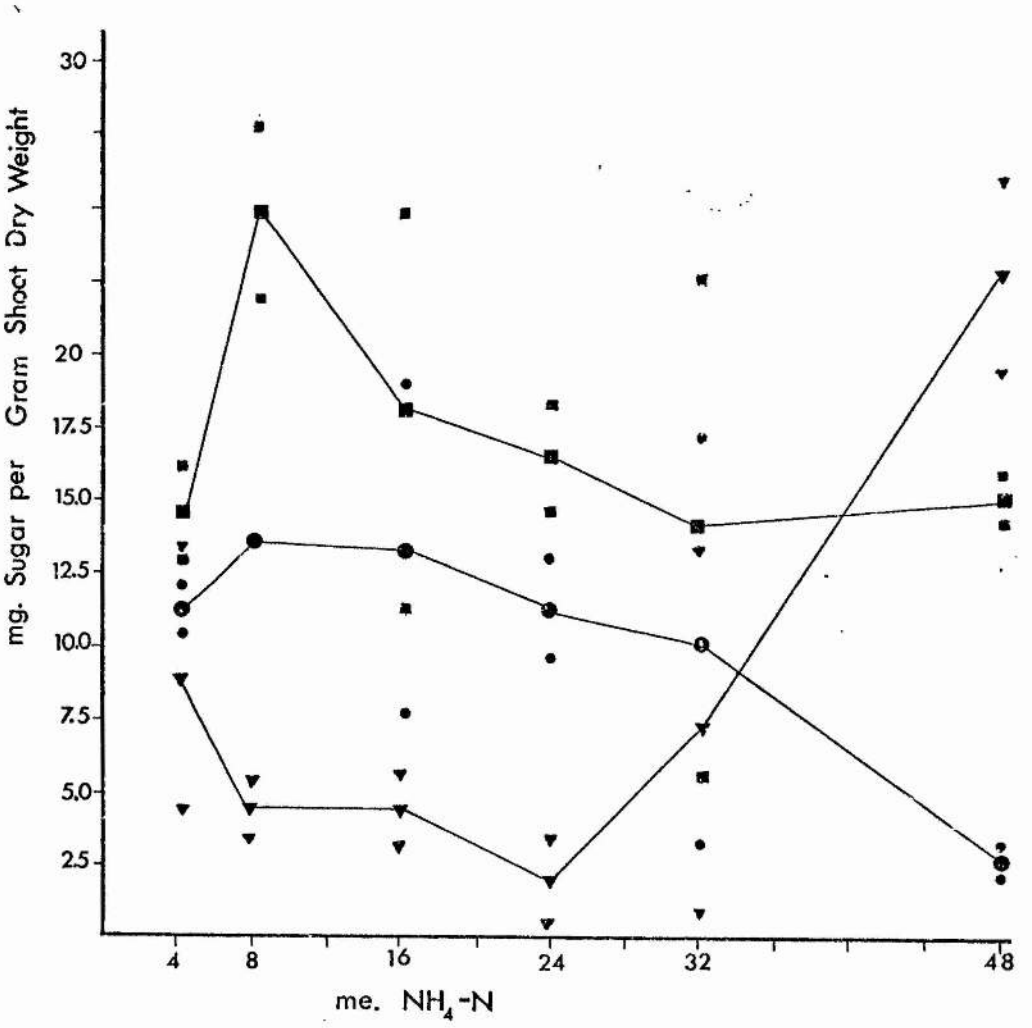


Figure 3.7c: The content of 3 sugars in A. tenuis shoots grown for 6 weeks in treatments of different NH_4 concentration. Each result is the mean of 2 replicates and is expressed as mg per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

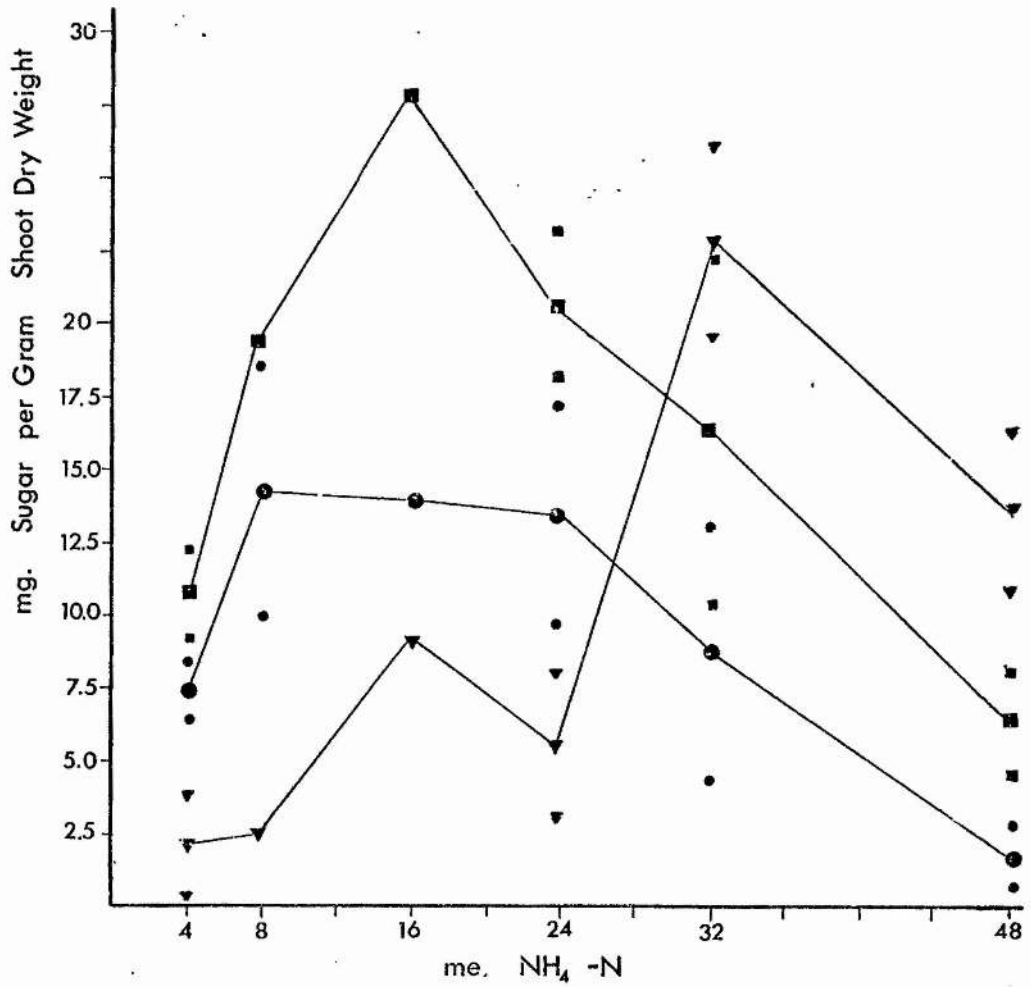


Figure 3.7d: The content of 3 sugars in F.rubra shoots grown for 6 weeks in 6 treatments of different NH_4 concentration. Each result is the mean of 2 replicates and is expressed as mg sugar per gram shoot dry weight. these means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

NH_4 level was approached. The peaks in these species around 16 me./l NH_4 were most likely due to the effects of ammonium toxicity which later may have been overcome so that fructose and glucose were no longer present in abundance. This effect was most noticable in L. perenne in all three sugars. In each of these species, the responses of glucose and fructose to increasing concentrations of NH_4-N were nearly identical. However, in A. tenuis and F. rubra, though the patterns of glucose and fructose response were similar in each species, the amounts of the two reducing sugars were very different. In both these last species, glucose was more abundant at all NH_4 levels. In A. tenuis, a higher glucose concentration is maintained even at the highest NH_4-N level.

The patterns shown by the non-reducing sugar sucrose were different from those shown by the reducing sugars. L. perenne, D.glomerata and A. tenuis all showed the same pattern of initial decrease in sugar concentration at low levels of NH_4 (from 4 to 8 me./l), followed by an increase as the highest NH_4 level was approached. All four species showed, at least to some degree, the previously mentioned peak at 16 me./l NH_4. This again was especially apparent in L. perenne. F. rubra showed an increase in sucrose concentration from 24 me./l NH_4, as did the other species, but the

concentration fell off from 32 me./l to 48 me./l NH_4.

When these effects of NH_4-N were analysed separately from the other two sets of treatments, the analysis of variance showed the following. In all three sugars, the variability due to the effects of the concentration of NH_4-N was highly significant ($p<0.001$). When fructose was considered separately, the differences between the species also accounted for a significant portion of the variability ($p<0.01$). Most of this variability was due to the very different response of L. perenne. On the basis of the least significant difference between species at a probability level of 5%, A. tenuis, F. rubra and D. glomerata did not differ significantly in their responses to NH_4-N. All three, however, were significantly different from L. perenne. The species also differed in the responses of glucose to NH_4 ($p<0.01$). In this case, again on the basis of L.S.D. at a probability level of 5%, A. tenuis and F. rubra were not significantly different. L. perenne and D. glomerata were not significantly different either. Lastly, none of the four species differed significantly in sugar response to NH_4. There was no significant interaction effect between species and treatment in any of the sugars.

The last third of the experiment was carried out with a view to examining any effects caused by

different proportions of NH_4-N and NO_3-N. The preliminary experiment, which demonstrated the different effects of nutrient level on soluble carbohydrate concentration between the species of oligotrophic environments and those of eutrophic environments, contained both NH_4-N and NO_3-N in the proportion of 2 me./l : 14 me./l respectively for the 100% nutrient concentration. The concentration of NH_4-N was 14.28% of the NO_3-N concentration at all levels. Since the differences between these two different sorts of plants may only have been demonstrable in the presence of a NO_3/NH_4 combination, it was necessary to have some treatments in which the total amount of nitrogen in me./l remained constant, but in which the relative amounts of NO_3-N and NH_4-N varied from treatment to treatment. A constant level of 24 me./l of nitrogen was chosen as this was half the maximum value used in the rest of this experiment, and not too different from the 16 me./l nitrogen value of the modified Hoagland's solution. The results are presented in the following tables followed by the same data in graphical form.

Table 3.4a-c: Influence of 24 me./l of nitrogen on the content of 3 sugars in 4 species of grasses when presented in 7 treatments with varying proportions of NH_4-N and NO_3-N. Two replicates of each species were grown for 6 weeks in treatments of otherwise identical nutrient solutions. Results are expressed as the mean of two replicates (each of which was analysed separately) in terms of mg sugar per gram shoot dry weight.

Table 3.4a: Fructose content

me./l NO_3-N:NH_4-N	D.glomerata	L.perenne	A.tenuis	F.rubra
0:24	12.5205	13.2533	11.1747	16.2381
4:20	7.1406	9.6006	40.5278	10.2918
8:16	4.1925	5.7836	8.1686	2.9572
12:12	5.4159	17.6203	14.0727	13.9693
16:8	6.6084	13.46404	11.5822	13.1405
20:4	3.9452	7.3236	10.5681	3.5913
24:0	13.7517	15.8469	19.0936	18.3512

Table 3.4b: Glucose content

me./l NO_3-N:NH_4-N	D.glomerata	L.perenne	A.tenuis	F.rubra
0:24	12.9935	17.1465	18.1208	24.4500
4:20	8.4935	9.8666	76.1203	12.0283
8:16	5.3577	3.9017	13.8588	6.2179
12:12	4.9836	18.8851	17.6223	17.1631
16:8	7.2784	12.1918	34.6481	14.3836
20:4	3.6575	2.4056	2.7096	5.2107
24:0	10.7377	15.4554	25.2228	19.4775

Table 3.4c: Sucrose content

me./l NO_3-N:NH_4-N	D.glomerata	L.perenne	A.tenuis	F.rubra
0:24	0.3509	1.4909	1.4302	2.6004
4:20	4.8317	2.2834	23.6005	1.6128
8:16	1.7495	2.6785	1.9012	7.3206
12:12	0.1561	0.1966	0.4244	2.0439
16:8	0.2341	0.4493	0.5388	2.0062
20:4	2.7371	10.3135	11.6804	15.7226
24:0	0.6066	0.5865	8.2363	1.8823

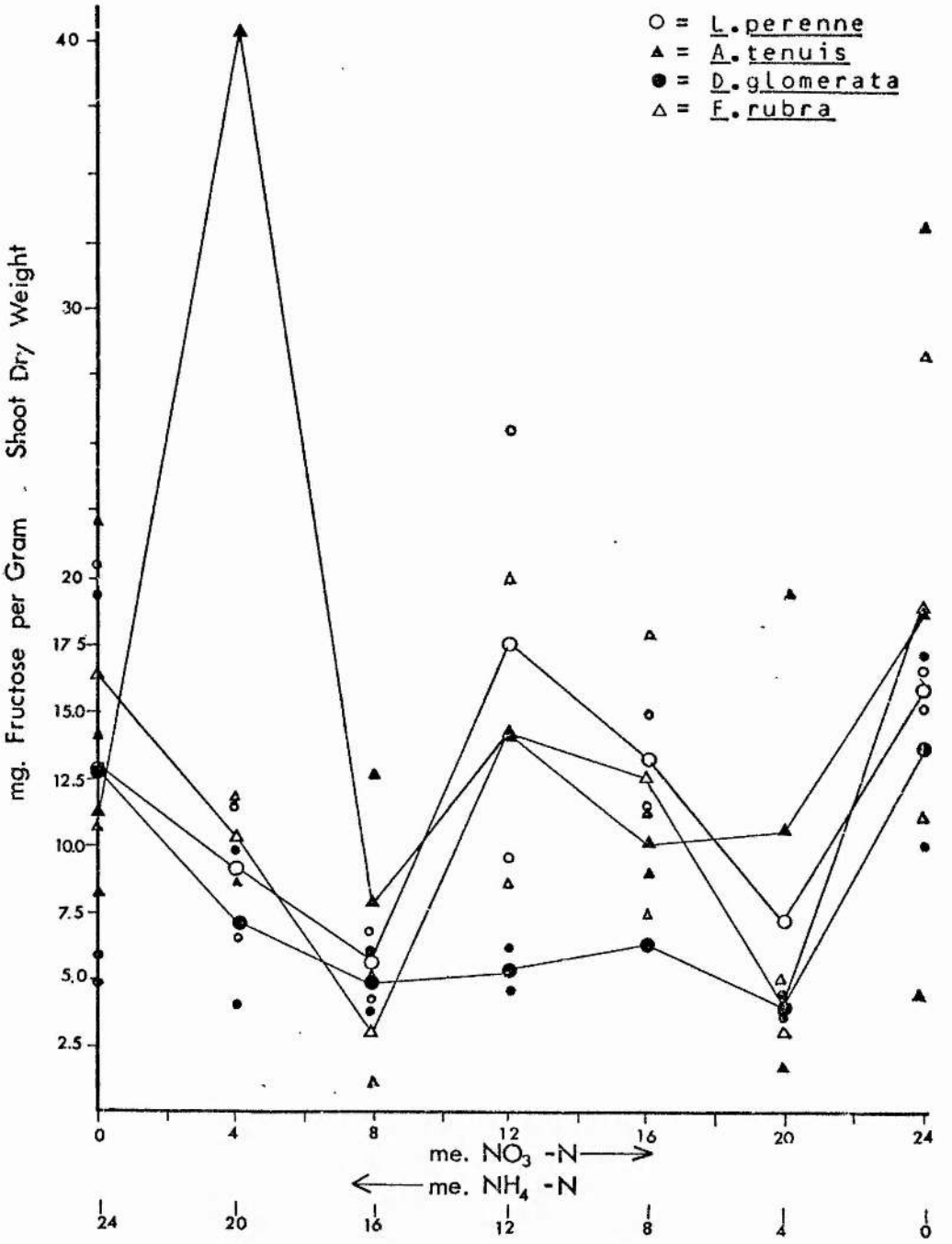


Figure 3.8a: Effect of 24 me./l nitrogen in 7 treatments of varied proportions of NO_3-N and NH_4-N on the fructose content of 4 species grown for 6 weeks in otherwise identical nutrient solutions. The amount of sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

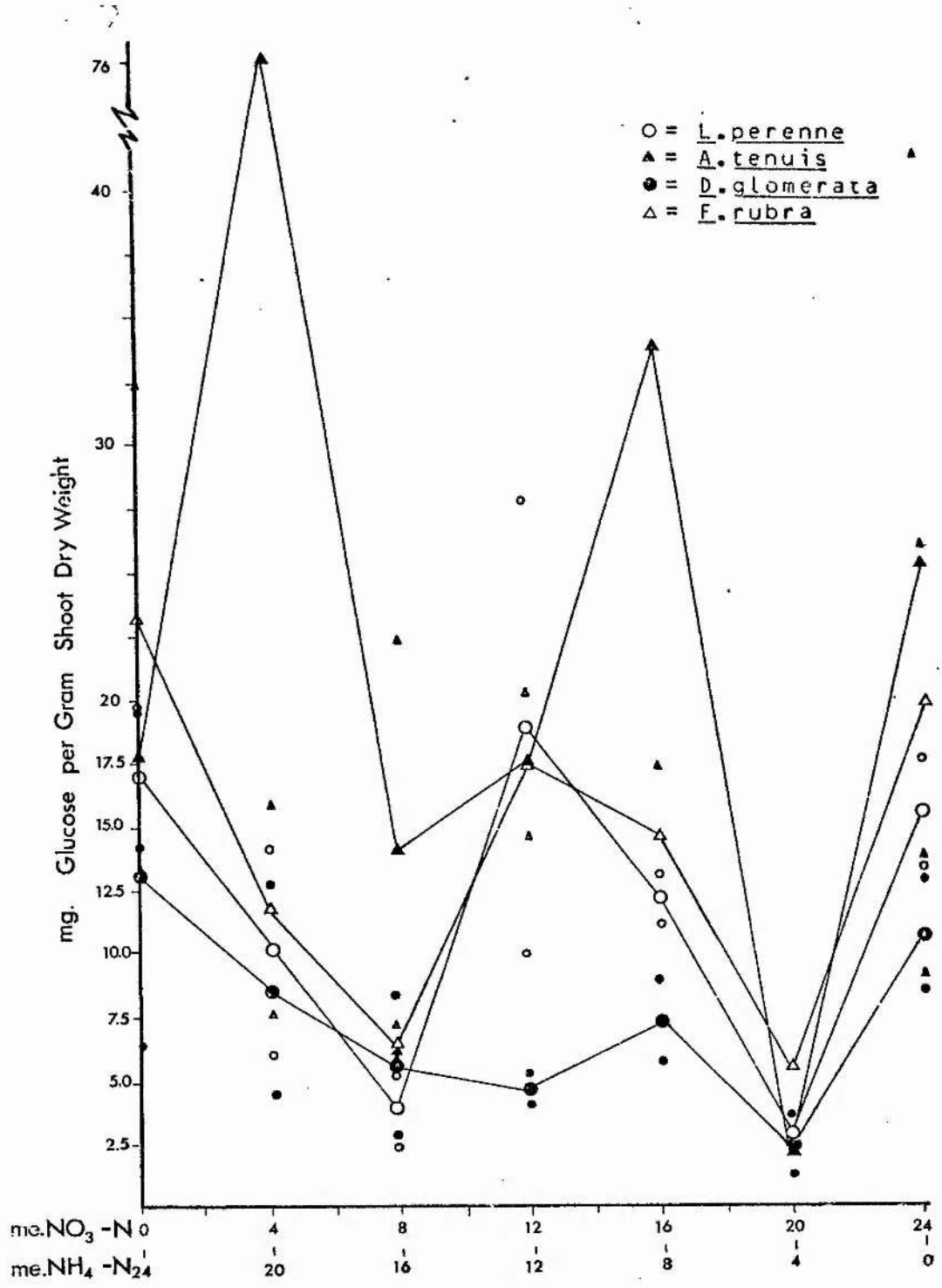


Figure 3.8b: Effect of 24 me./l nitrogen in 7 treatments of varied proportions of NO_3-N and NH_4-N on the glucose content of 4 species grown for 6 weeks in otherwise identical nutrient solutions. The amount of sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the 2 replicates are connected by lines, and the actual values of the replicates are shown.

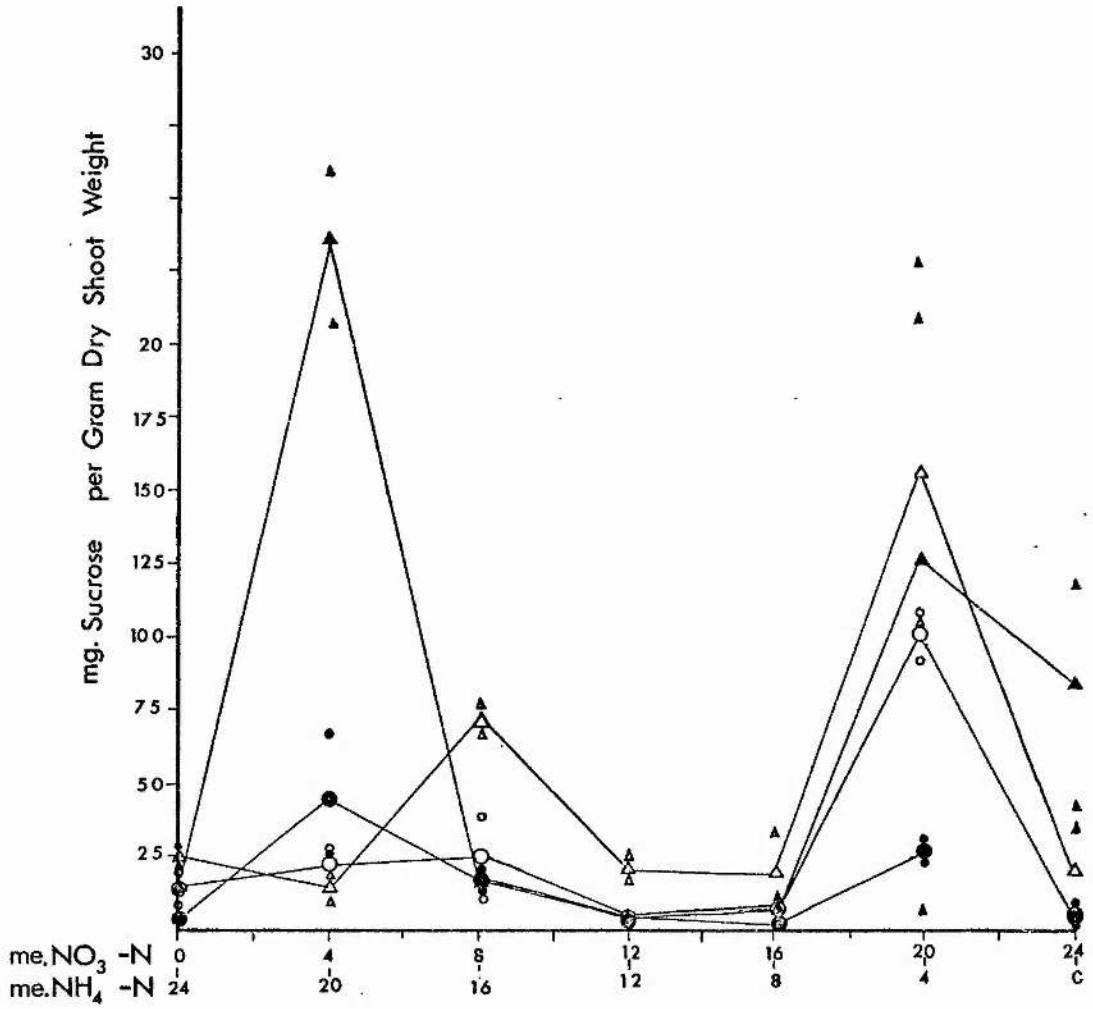


Figure 3.8c: Effect of 24 me./l nitrogen in 7 treatments of varied proportions of NO_3-N and NH_4-N on the sucrose content of 4 species grown for 6 weeks in otherwise identical nutrient solutions. The amount of sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○ = L. perenne ● = D. glomerata
▲ = A. tenuis △ = F. rubra

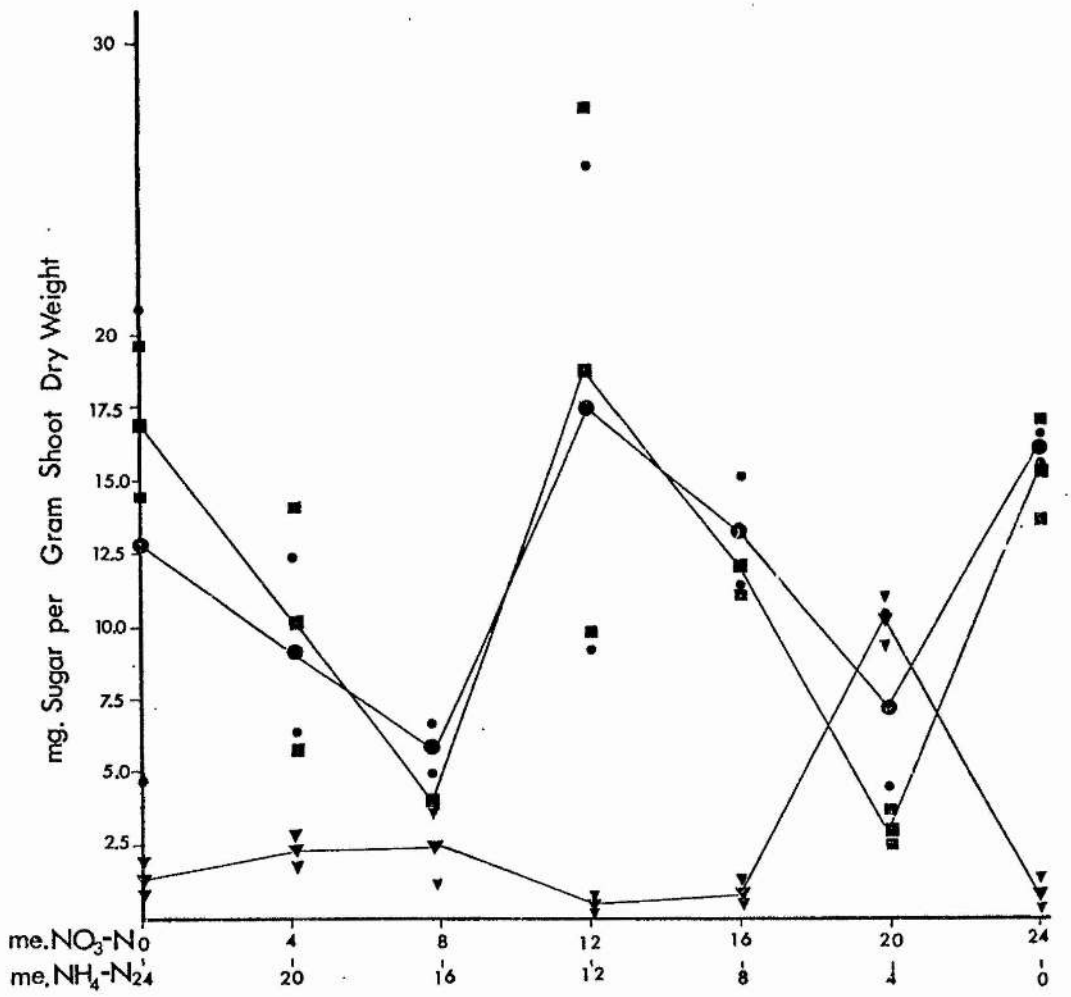


Figure 3.9a: The content of 3 sugars in L.perenne shoots grown for 6 weeks in 7 treatments of complete nutrient solution with a nitrogen concentration of 24 me./l in which the proportions of NO_3-N and NH_4-N varied from treatment to treatment. Each result is the mean of 2 replicates and is presented in terms of mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

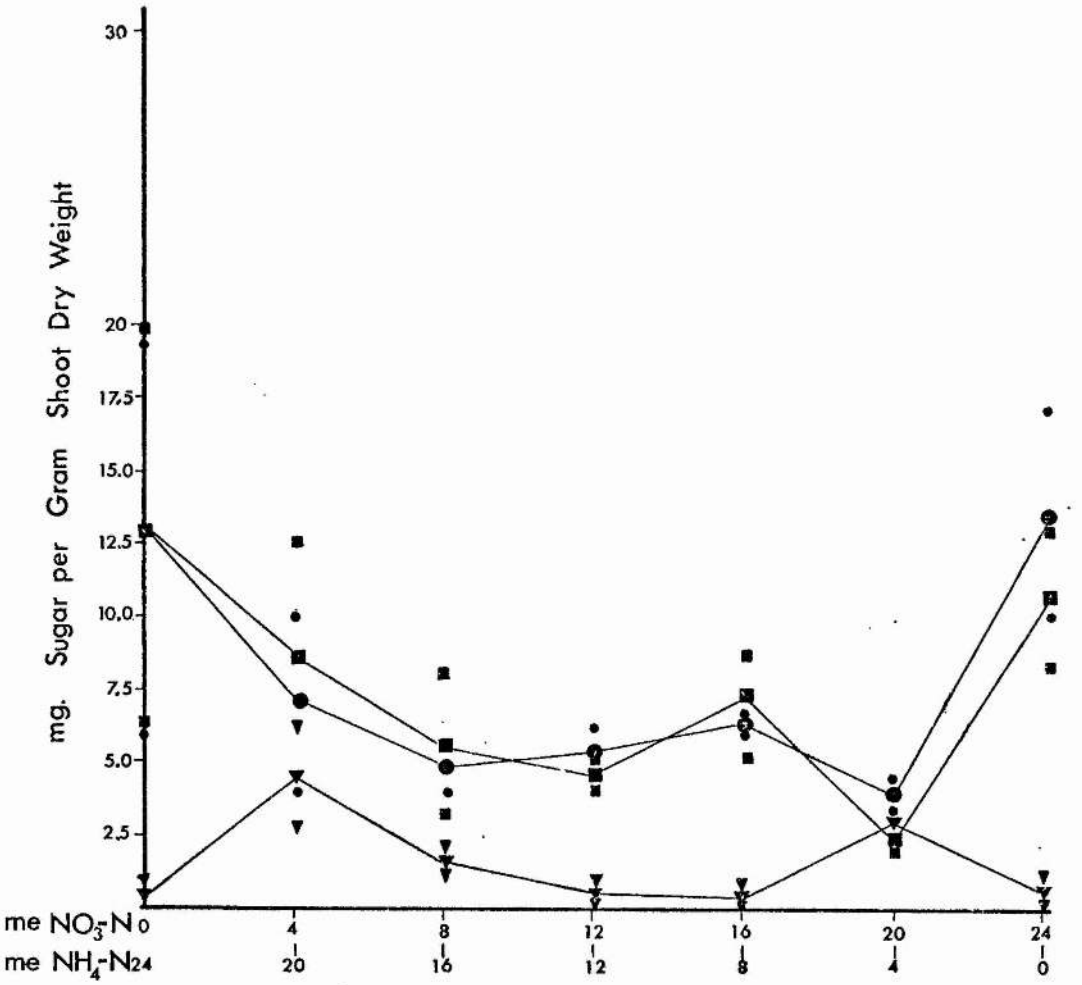


Figure 3.9b: The content of 3 sugars in D.glomerata shoots grown for 6 weeks in 7 treatments of complete nutrient solution with a nitrogen concentration of 24 me./l in which the proportions of NO_3-N and NH_4-N varied from treatment to treatment. Each result is the mean of 2 replicates and is presented in terms of mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

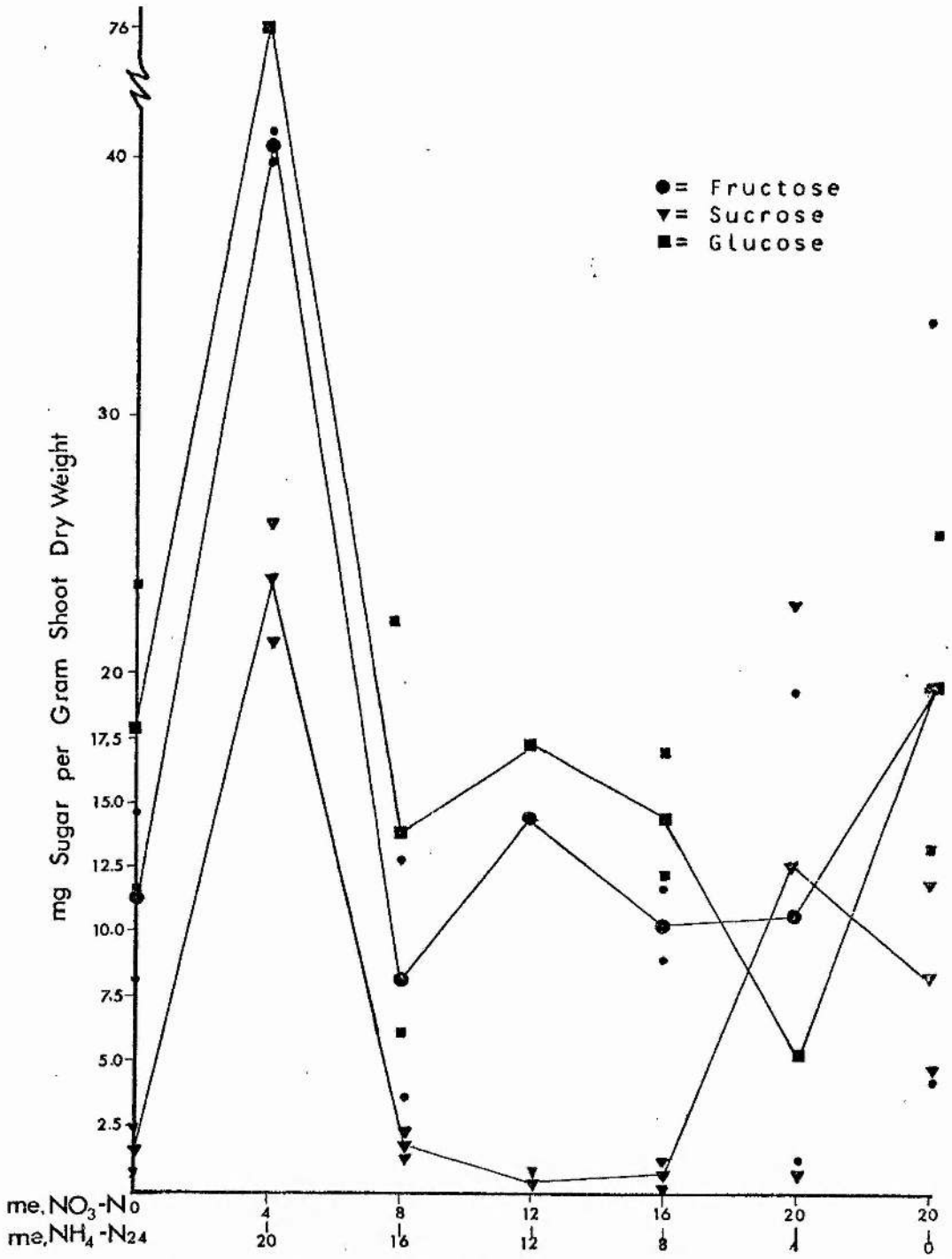


Figure 3.9c: The content of 3 sugars in A. tenuis shoots grown for 6 weeks in 7 treatments of complete nutrient solution with a nitrogen concentration of 24 me./l in which the proportions of NO_3-N and NH_4-N varied from treatment to treatment. Each result is the mean of 2 replicates and is presented in terms of mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

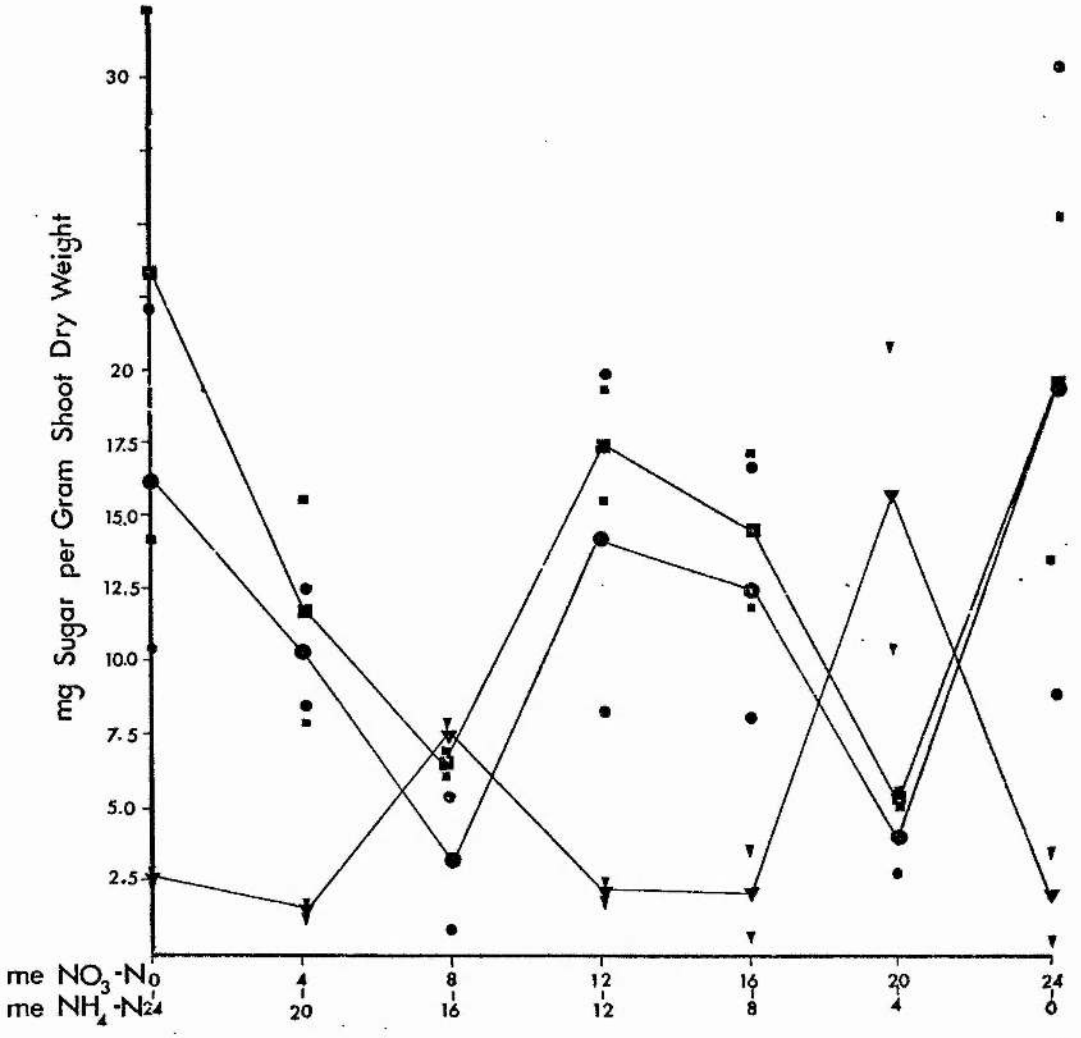


Figure 3.9d: The content of 3 sugars in F.rubra shoots grown for 6 weeks in 7 treatments of complete nutrient solution with a nitrogen concentration of 24 me./l in which the proportions of NO_3-N and NH_4-N varied from treatment to treatment. Each result is the mean of 2 replicates and is expressed in terms of mg sugar per gram shoot dry weight. These means are connected by lines, and the actual values of the replicates are shown.

● = Fructose
▼ = Sucrose
■ = Glucose

It is apparent from these results that fructose and glucose show a general pattern consisting of high sugar concentration both at high ammonium and high nitrate levels. The sugar concentration was also high in the treatment in which NO_3-N and NH_4-N were in equal proportions. When the species are examined separately, the responses of L. perenne and F. rubra were very similar, and each species showed the same pattern of sugar accumulation in both sugars. D. glomerata was quite different in that its sugar levels were very low compared to the other species. Additionally, the large increase in sugar concentration in response to the 12:12 me./l NO_3-N:NH_4-N treatment was absent, and the small increase which did occur was favoured by a higher NO_3-N:NH_4-N ratio (ie. 16:8). A. tenuis showed quite a different response to the treatments in which the NO_3-N:NH_4-N ratios were low. A. tenuis responded to the 4:20 ratio with a massive accumulation of both sugars; in this treatment all three of the other species showed a decrease in sugar concentration. In A. tenuis, as in D. glomerata, glucose increased in concentration in the 16:8 treatment rather than the lower 12:12 treatment, a pattern common to the other species.

The response of sucrose to these treatments was different in that there were essentially only 2 treatments in which there was an increase in sugar

concentration (4:20 me./l NO_3-N:NH_4-N and 20:4 me./l NO_3-N:NH_4-N). The sucrose response to the very high NO_3-N and NH_4-N and the middle range treatments was very low compared to that in the reducing sugars. In all species except A. tenuis, sucrose concentration was higher in the 20:4 me./l NO_3-N:NH_4-N treatment than in the 4:20 treatment. In general, the amount of sugar per gram shoot dry weight was greater for the species from oligotrophic environments than those from eutrophic environments. With the exception of the 4:20 NO_3:NH_4 treatment of A. tenuis, sucrose showed the opposite pattern of response to that of the reducing sugars: sucrose accumulated when the reducing sugars decreased.

An analysis of variance for this set of treatments showed that the variability due to treatment effects was significant in all three sugars. The variability due to species difference was significant at the 5% probability level for fructose, the 0.1% level for glucose and the 1% level for sucrose. There were significant species/treatment interactions ($P<0.01$) in the responses of glucose and sucrose to these treatments. L.S.D at 5% showed that in the fructose response, D. glomerata was significantly different from the group formed by A. tenuis, F. rubra and L. perenne. This was due to the consistent, very low, levels of fructose in D. glomerata. With the response of glucose,

A. tenuis was significantly different from the other three species. The variability in this sugar was largely due to the very different response of A. tenuis. The same was true of sucrose.

The separate statistical analyses for each part of this experiment have already been presented. It was possible to use an analysis of variance to handle each of these sugars, the 4 species, 19 treatments and 2 replicates all at once. Due to the experimental design a simple analysis of variance was not adequate to analyse the data. The method used was "Generalised Linear Interactive Modelling". Particulars on this method may be found in "GLIM 2 Manual" from the Computing Laboratory, University of St. Andrews.

Table 3.5a-c: GLIM Analysis of variance of the effect of kind and quantity of nitrogen supplied to 4 grass species on the concentration of 3 sugars. (mg sugar per gram shoot dry weight. Data from tables 3.2, 3.3, 3.4.

Table 3.5a: Fructose content

Source of Variance	Sum of Squares	d.f.	M.S.	F	
Species	6.5	3	2.16	3.6	*
NO_3-N	8.73	1	8.73	14,6	***
NH_4-N	1.21	1	1.21	2.1	NS
Replicates	0.06	1	0.06	10.0	NS
Species Replicates	0.34	3	0.11	5.3,	NS
Species NO_3-N	0.27	3	0.09	6.64	NS
Species NH_4-N	0.57	3	0.19	3.1	NS
Residual	81.36	136	0.598		
TOTAL	99.04	151			

17.85% of the variability is explained
* P<0.05 *** P<0.001
NS - not significant

Table 3.5b: Glucose content

Source of Variance	Sum of Squares	d.f.	M.S.	F	
Species	16.5	3	5.5	4.46	**
NO_3-N	22.6	1	22.6	18.34	***
NH_4-N	0.3	1	0.3	4.1	NS
Replicates	0.2	1	0.2	6.2	NS
Species replicates	0.3	3	0.1	12.3	*
Species NO_3-N	1.5	3	0.5	2.5	NS
Species NH_4-N	2.0	3	0.7	1.6	NS
Residual	167.6	136	1.2		
TOTAL	211.0	151			

20.56% of the variability is explained
* P<0.05, ** P<0.01, *** P<0.001
NS - not significant

Table 3.5c: Sucrose content

Source of Variance	Sum of Squares	d.f.	M.S.	F	
Species	3.36	3	1.12	2.4	NS
NO_3 -N	0.06	1	0.06	7.9	NS
NH_4 -N	14.82	1	14.82	31.1	***
Replicates	0.02	1	0.02	23.8	NS
Species replicates	0.24	3	0.08	5.9	NS
Species NO_3 -N	0.97	3	0.32	1.5	NS
Species NH_4 -N	0.21	3	0.07	6.8	NS
Residual	64.77	136	0.47		
TOTAL	84.45	151			

23.3% of the variability is explained
*** P<0.001
NS - not significant

Summary and Discussion of Results

The results of this experiment, which examined the response of soluble carbohydrates to different nitrogen concentrations of L. perenne, D. glomerata, A. tenuis and F. rubra, can be summarised as follows:

(1) As the level of NO_3-N increased, the reducing sugar content of all four species decreased. The effect of NO_3-N treatment on reducing sugar content was statistically significant as was the variability due to species differences. Further, there was some variability due to species treatment interaction, (with fructose, $p<0.02$ and with glucose, $p<0.05$) showing that there may be small differences in the manner in which these species respond to NO_3-N.

(a) In L. perenne and D. glomerata, this decrease was rapid between 4 me./l NO_3-N and 8 me./l NO_3-N followed by a more gradual decrease. On the basis of least significant differences (L.S.D.), there was a significant difference between L. perenne and D. glomerata at the 0.05 probability level for fructose response and no significant difference between these species in the response of glucose.

(b) In F. rubra and A. tenuis, this decrease was gradual throughout the range of treatments. When considering the L.S.D. ($P<0.05$), there was no

significant difference in the responses of either fructose or glucose between A. tenuis and F. rubra.

(2) The reducing sugar content of F. rubra and A. tenuis was, in general, lower than that of L. perenne and D. glomerata especially in the 4 me./l NO_3-N treatment. Any statistically significant differences in the response of reducing sugars to increasing NO_3-N between species appear to be due mainly to quantitive differences rather than to differences in pattern of response.

(3) The response of sucrose to increasing NO_3-N was very different in the four species compared to the responses of the reducing sugars. Again, the effect of NO_3 treatment on sucrose content was statistically significant, and the variability due to species differences was moderately significant ($P<0.05$). There was also a significant treatment/species interaction effect ($P<0.05$), indicating that the species respond to increasing NO_3-N in different ways.

(a) These species showed a decrease in sucrose from 4 me./l to 8 me./l NO_3-N, but unlike the responses of the reducing sugars, the maximum sucrose concentration was not at the 4 me./l NO_3-N level. The NO_3-N level of maximum sucrose

concentration was 16 me./l for L. perenne, 24 me./l for D. glomerata and A. tenuis, and 48 me./l for F. rubra.

(b) A. tenuis and F. rubra maintained a higher sucrose level at higher NO_3-N concentrations than the other two species. F. rubra responded with gradual increase in sucrose concentration from the 8 me./l NO_3-N treatment to the 48 me./l NO_3-N level.

If these species differ in the pattern of response of sugar concentration as the preliminary experiment suggested, the effect, at least with the reducing sugars, is probably not due solely to differences in NO_3-N. NO_3-N does, however, have a significant effect on sugar concentration in these species, but the effect appears to be mainly one of differing concentrations rather than different patterns of response.

(4) As the level of NH_4-N increased, the response of the reducing sugars to treatment level was highly significant ($P<0.001$). The variability due to species difference was also significant ($P<0.01$), but appeared to be accounted for mainly by the very different response of L. perenne. As with increasing NO_3-N, there was some interaction between species and treatments ($P<0.05$ for fructose, $p<0.02$ for glucose)

which may indicate different responses in the different species, but which was most likely due to the vastly different response of L. perenne.

(a) The response of the reducing sugars to increasing NH_4-N was very similar in A. tenuis and F. rubra, and on the basis of L.S.D. at $P<0.05$, they were not significantly different. In both species, there was an increase in reducing sugar concentration from the 4 me./l to the 8 me./l NH_4-N level. The sugars then reached maximum concentration between 8 me./l and 16 me./l NH_4-N which was followed by a decrease in concentration approaching 48 me./l NH_4-N.

(b) The response of fructose to increasing NH_4-N was significantly different between D. glomerata and L. perenne on the basis of L.S.D. at $P<0.05$, but their glucose response was not significantly different. The principal differences were the much higher sugar concentrations of L. perenne, and the decrease in sugar concentration of this species between the 4 me./l and 8 me./l levels. D. glomerata showed an increase in sugar concentration over this range. These species exhibited sugar maxima over the range 8me./l and 32 me./l NH_4-N. They both showed an increased reducing sugar concentration over the range 32 me./l to 48 me./l

NH_4-N.

(5) With increasing NH_4-N, there was a highly significant ($P<0.001$) difference between treatments in the response of the non-reducing sugar sucrose. The variability due to species differences was not significant, and there was no significant species/treatment interaction.

(a) The response of sucrose in all four species was one of initial decrease in sugar concentration at low levels. Again this was most distinct in L. perenne and was followed by the previously mentioned peak at 16 me./l NH_4-N, which was present in all the species to some degree. In L. perenne, A. tenuis and D. glomerata this was followed by a rapid increase to maximum sugar concentration at 48 me./l NH_4-N.

(b) F. rubra was an exception in that its maximum was reached at 32 me./l NH_4-N and was followed by a decrease in sugar concentration as 48 me./l NH_4-N was approached.

Again, it is seen that NH_4-N has a significant effect on sugar concentration in these species. Additionally, a significant amount of variability in the response of the reducing sugars to NH_4-N level is due to species differences. As was the case with sugar

response to NO_3-N, it appears that these differences are mainly due to quantitative differences, and except perhaps with glucose, not due to different patterns of response between the species. NH_4-N on its own does not appear to account for the responses observed in the preliminary experiment.

(6) The variability in the response of the reducing sugars to 24 me./l nitrogen in treatments of different proportions of NO_3-N and NH_4-N was seen to be due to both significant treatment and species differences. There was no significant species/treatment interaction in fructose response, but this interaction was significant in the glucose response.

(a) With the exception of A. tenuis, which showed an initial increase over the 0:24 me./l - 4:20 me./l NO_3-N:NH_4-N treatments, the general reducing sugar response of all the species to these treatments was a decrease over the range 0:24 me./l NO_3-N:NH_4-N, followed by a peak in the region of 8:16 me./l NO_3-N:NH_4-N and a final, rapid increase from 20:4 to 24:0 me./l.

(b) The significance of the variability due to species differences was examined using L.S.D. at $P<0.05$, and was seen to be due mainly to the very low response of D. glomerata in the case of

fructose. In glucose, the significance was due to the very great response of A. tenuis.

(7) The response of sucrose to these treatments was very nearly the opposite of the reducing sugar response. The variability due to treament effects was highly significant ($P<0.001$), and that due to species differences was significant at the 1% probability level. There was also a significant ($P<0.01$) species/treament interaction.

(a) L.S.D. at $P<0.05$ revealed that A. tenuis and F. rubra were not significantly different. The sucrose response in these species revealed two peaks of sugar concentration. A. tenuis showed a peak at the 4:20 me./l level, F. rubra at 8:16 me./l NO_3-N:NH_4-N. They both showed minimum concentration over the 12:12 me./l to 16:8 me./l range, followed by another peak at 20:4 me./l and a decrease approaching 24:0 me./l NO_3-N:NH_4-N.

(b) The same two peaks were observed in L. perenne and D. glomerata, though the concentrations of sucrose in these two species were less over this range of treatments than in either A. tenuis or F. rubra. L.S.D. at $P<0.05$ showed that F. rubra, L. perenne and D. glomerata were not significantly different.

Once again, the results of this experiment are different from those of the preliminary experiment, but the treatments have had a significant effect on sugar concentration in these species, and the species have shown different responses. Again, these differences are mainly quantitative.

Discussion

In spite of the fact that the results of the preliminary experiment appear not to be due simply to the effect of the kind and amount of nitrogen supplied, several interesting responses have been revealed. Nitrate-nitrogen causes a definite pattern of decreasing reducing sugar concentration, and although there may be significant differences between the species quantitatively, the patterns of the species are much the same. Apart from the evidence that L. perenne and D. glomerata contain a greater concentration of reducing sugars in their shoots than either A. tenuis or F. rubra, NO_3-N does not, apparently, bring out the differences between species adapted to oligotrophic and eutrophic environments. The same is true when the response of sucrose is examined, although the tendency is for sucrose to increase with increasing NO_3-N. When NH_4-N is increased in the different treatments, there is even less difference between the reducing sugar

response of the species. (L. perenne has an exceptional response to NH_4-N).

Experiments have shown that in a number of species the form of nitrogen supply can cause pronounced effects on the chemical composition and growth of plants (Kirkby, 1968). Futhermore, it has been shown that different species react differently to increases in nitrogen fertilisation. For example, Nowakowski and Cunningham (1966) found that nitrogen fertilisation considerably decreased the soluble carbohydrates in D. glomerata (consistent with the results presented here). Adegbola and McKell (1966) reported that F. rubra L. accumulated more sugar under conditions of high fertility than under those of low fertility. In another experiment, they found that the total soluble carbohydrates in Lolium multiflorum decreased with increasing nitrogen above 100ppm. Disaccharides in oats have been shown to increase with deficiencies of nitrogen and phosphorus (Lawanson et.al.,1975). On the other hand, Adegbola and McKell (1966) have found that in coastal Bermuda grass (Cynodon dactylon (L.),Pers.), a forage crop, the reducing sugar content increases with increasing rates of N-fertilisation. However, in barley, low level of nitrogen raised the level of total sugar and the ratio of sucrose to reducing sugars. Green and Beard (1969) found that nitrogen treatments

did not show any attributable responses in reducing sugars or sucrose, nor did these sugars follow any obvious seasonal trends. In Agrostis palustris Huds. they did, however, find that oligosaccharides other than sucrose decreased in concentration as nitrogen increased. It is quite obvious then that the responses of carbohydrates to nutrition is dependent upon many factors, not the least of which is the unique reactions of the particular species.

Some of the factors contributing to the response of soluble or metabolic sugars observed in plant tissues may now be discussed preceded by an extremely simplified sketch of plant organisation. At the most basic level, the higher plant could be thought of as being composed of reduced carbon units. The fueling of biophysical and/or biochemical changes, as well as the maintenance of the existing plant body, require chemical energy supplied by these units. Respiration oxidises some of these reduced carbon units and returns them to CO_2 through oxidation. These units, the immediate products of photosynthesis, may alternatively be involved in the synthesis of sugars, amino acids, and other molecules, which may be translocated immediately mainly as sucrose, amino acids or amides, respectively. Storage may then take place for later utilisation. Mineral salts are meanwhile taken up

throught the root system. Some of these salts may pass through the transpiration stream to the leaves to be involved in synthesis of, for example, amino acids. The fates of some salts may be very different: they may be passively bound on the way through the transpiration stream; they may be actively taken into cell vacuoles or be involved in cellular metabolism in the cytoplasm. Nitrate, for instance, may be reduced and incorporated into amino acids in the roots. As the life of the plants photosynthetic machinery is limited, in order to maintain the same photosynthetic rate, new photosynthetic organs must be produced involving directly or indirectly the plants whole metabolic machinery. Thus, carbohydrate content is related to growth.

Indeed, Brown and Blaser (1965) have shown that under conditions which promote rapid growth, the plant's carbohydrate content was very low and/or was maintained at a low level. This led them to postulate that, if soluble carbohydrates represent energy and reserve materials, there is, in general, an inverse relationship between the accumulation of reserve soluble carboydrates and growth rate in perennial forage crops. If this is true, then the accumulation of reserve soluble carbohydates will depend on a balance between the energy needs of a plant for growth and the

supply of energy through photosynthesis. Additionally, soluble carbohydrates would be expected to accumulate if photosynthesis was relatively high during conditions of slow growth. Waite and Garrod (1959) found that the slower growing rye-grass had the highest sucrose levels of the four species which they studied. This effect of growth rate may explain the results of the preliminary experiment. Jones, Griffith and Walters (1965) assumed the depression in soluble carbohydrate concentration which they found in a study of L. perenne and D. glomerata was due largely to the acceleration in growth rate which accompanied nitrogen fertilisation. As to the relative importance of reducing and non-reducing sugars, Green and Beard (1969) associated maximum reducing sugars with rapid vegetative growth, maximum sucrose with differentiation and maximum "reserve polysaccharides" with the "rest period" prior to secondary growth. Adegbola and McKell (1966) associated high concentrations of reducing sugars with increased stimulation of growth in Coastal Bermuda grass, and found that at the highest levels of fertilisation this grass apparently retained most of the newly synthesised carbohydrate in the leaves as reducing sugars.

Another factor involved in this is protein synthesis. This is one of the principal uses of carbohydrates in the plant. Therefore, assuming

photosynthesis continues, the synthesis of protein as well as structural carbohydrates from soluble carbohydrates would cause a reduction in the soluble and reserve carbohydrates of grasses receiving fertilisation. Conversely, if there was a decrease in the synthesis of organic nitrogen, an accumulation of carbohydrate would be expected. Additionally, if proteins were being broken down, the resulting carbon skeletons could spare the available carbohydrate reserves or replace them entirely. It is also reasonable to assume, too, that any factor retarding nitrate assimilation would affect carbohydrate consumption.

Certain of the different responses of soluble carbohydrates to NO_3-N and NH_4-N nutrition may be anticipated. Whether nitrate or ammonium is being assimilated by the plant, carbohydrates are required as a source of carbon skeletons. They are also needed as the substrate for the supply of energy needed for reductive amination. When NO_3-nitrogen is being used by a plant, some of this carbohydrate produced by photosynthesis is needed for the reduction of NO_3 to NH_4. Hence, the production of NH_4 by the assimilation of NO_3 is related metabolically to the oxidation of reduced co-enzymes. On the basis of this, it would seem logical to expect a lower concentration of sugars in

NO_3-N fed plants than in those fed with some range of NH_4-N as the assimilation of NH_4 does not side-track photosynthetic energy in the same way as NO_3 utilisation. However, NH_4 assimilation is a comparitively less-controlled source of NH_4 production (Kirkby, 1968). Assuming that an adequate carbohydrate supply is present, NH_4 assimilation should be able to proceed more rapidly than the assimilation of NO_3, thus leading to the high proportion of organic nitrogen compounds in NH_4 fed plants compared to those which are fed nitrate (Nowakowski and Cunningham, 1966). Therefore, this rapid assimilation would be expected to deplete carbohydrate reserves. The uptake of ammonia should also reduce carbohydrates due to the use of organic acids in detoxification. In fact, Kirkby (1968) found high levels of sugars associated with NH_4 nutrition. The NH_4-N treatments in this chapter also showed quite high levels of sugar, particularly in the reducing sugars and in the higher NH_4-N treatments of sucrose. This could be due to concentration effects associated with a depression of growth induced by NH_4. It is generally known that plants fed only NH_4-N do not grow as well as those given NO_3-N. These plants tend to contain lower concentrations of inorganic cations (Kirkby, 1968), and elements which were originally absorbed as anions are in greater proportion than in

NO_3-N fed plants.

These different carbohydrate responses are directly related to the survival of species in the field. The stimulation of vegetative growth as a result of fertilisation tends to cause the creation of more photosynthetic area, which in turn leads to more carbohydrate production. However, fertilised plants respire even more carbohydrates, and they have a greater need for synthetic processes including structural development. This depletion of carbohydrate reserves caused by additional supplies of nitrogen can hinder plant regrowth. It has been found that application of nitrogen weakened individuals of D. glomerata and F. pratensis so that extensive damage occurred following difficult winter periods (Huokuna, 1974). A study of Poa pratensis L. ("Merion" Kentucky bluegrass) revealed that the response to this turfgrass to stresses such as heat, draught and disease was improved when the carbohydrate concentration was highest (Watsche and Waddington, 1974). Depletion of carbohydrates caused a physiologically weakened condition. It follows, then, that a species with a low growth rate and reasonable carbohydrate reserves would be better able to survive the stresses of a natural environment.

These insights must be applied to the results of

this experiment. The factors favouring a high soluble carbohydrate content appear to be a low amount of growth (if photosynthesis is maintained), very high rates of fertilisation (probably causing a depression of growth due to high NO_3 or NH_4 concentrations which does not affect the soluble carbohydrate production), nitrogen and phosphorus deficiencies, and a low rate of amino acid/protein synthesis. Those favouring low concentrations of soluble carbohydrates have been related to increased nitrogen fertilisation, decreased photosynthesis, growth rates rapid enough to use the immediate products of photosynthesis as they are produced, increased rates of protein/amino acid synthesis and a rapid assimilation of NH_4-N.

It was seen in the first chapter that the four species used in this study differed in both their growth rates and in their maximum potential response, measured in mg shoot dry weight, to nutrient level. These factors, then, are likely to contribute to the response of soluble carbohydrate concentration to nitrogen level. When the NO_3-N phase of this experiment was examined, it was seen that the three sugars studied accumulated at low nitrate levels in all four species. This was probably due to the restriction of growth due to nitrogen deficiency. The four species each showed the greatest increase in dry weight production between

the two lowest nutrient levels. This rapid phase of growth would require the utilisation of much of the soluble carbohydrate in the plants. This is further supported by the observation that the species which showed the greatest decrease in reducing sugars between the lowest NO_3-N levels, L. perenne and D. glomerata, each showed the greatest increase in dry weight over the two lowest complete nutrient levels in the growth studies. This effect of nitrate-stimulated growth probably also accounted for the continued decline in reducing sugar concentration up to the 24 me./l NO_3-N level. After an initial decrease in concentration, sucrose accumulated from 8 me./l to 24 me./l NO_3-N. This would be expected if growth was continuing and if the reducing sugars were being used. Above 24 me./l NO_3-N, reducing sugar concentration was still very low, and there was a tendency for sugar accumulation at the higher NO_3-N level. This was accompanied by a general decrease in sucrose concentration. This, combined with the evidence from Chapter 2 that above 100% Hoagland's solution (containing 16 me./l N) growth rate as well as yield on a dry weight basis decreases substantially, indicates that above 24 me./l NO_3 soluble sugar concentration is influenced by depression of growth due to the high concentration of nitrate, which was accompanied by a decrease in the production of soluble

carbohydrate. The response of these three sugars to increasing NO_3-N is not markedly different in each of the four species.

The response of these same sugars to increasing NH_4-N was quite different. A. tenuis, D. glomerata and F. rubra showed accumulation of reducing sugars with increasing NH_4-N up to concentrations between 16 and 24 me./l NH_4-N and 24 me./l NH_4-N. Above this NH_4-N concentration, reducing sugar concentration decreased rapidly. This is difficult to account for solely on the basis of growth rates studied using complete nutrient solutions. It is possible that the very low initial concentrations of reducing sugars in these species was due to the very rapid assimilation of NH_4-N compared to that of NO_3-N, and at very low concentrations, reducing sugars are used immediately. The initial decrease in fructose and glucose seen in L. perenne probably indicates that this species responds to a different range of ammonium-nitrogen than the other species. The general trend of sucrose response is one of initial decrease in concentration followed by an accumulation with increasing NH_4-N. The accumulation is quite marked above 24 me./l NH_4-N. Again, this may be related to depression of growth at high NH_4-N concentrations combined with a decrease in carbohydrate production. At this point, the results of the NO_3-N/NH_4-N treatments

are even more difficult to explain. As soluble carbohydrate content, not production, was measured, it would not be wise or constructive to speculate much further on the causes of the response of soluble sugars here. The following chapter discusses the amino acid concentrations measured in these species in response to the same treatments. Further discussion of the possible causes of these particular sugar responses will be postponed to include any new insight gained by knowing the amino acid concentrations at these various nutrient levels.

EFFECTS OF SOLUTIONS OF DIFFERENT NITRATE AND PHOSPHOROUS CONCENTRATIONS ON SOLUBLE CARBOHYDRATES

Since the nitrate/ammonium experiment failed to account for the results of the first complete nutrient experiment, the same four species (*D. glomerata*, *L. perenne*, *F. rubra* and *A. tenuis*) were grown in solutions which varied in phosphorus and nitrate concentrations. This experiment was an attempt to find out if the results of the first experiment (in which a range of total nutrient concentrations revealed differences in sugar accumulation between the species from oligotrophic environments and those of eutrophic environments) could be explained by the reactions of these species to varying phosphorus levels. It would also show if a nitrate/phosphate combination could account for the results. This experiment would also indicate if species from oligotrophic environments react differently physiologically, from species of eutrophic environments to different phosphate concentrations in ways which would affect sugar and/or amino acid concentration.

There were several reasons why the study of the reactions of soluble carbohydrates to phosphate concentration was important to this investigation. Phosphorus is an important plant nutrient essential for

energy transfer in metabolism. Like nitrogen, the amount of phosphorus can vary from one soil to another. Additionally, it is known that species differ considerably in their growth responses to the supply of phosphorus in the soil (Pigott and Taylor, 1964; Rorison, 1968). As it has been shown that growth is related to soluble carbohydrate content, it was reasonable to expect different amounts of phosphorus in nutrient solutions to affect soluble sugar concentration in different species.

Method

A. tenuis, F. rubra, D. glomerata and L. perenne were also used in this experiment. Seeds of the four species were sown in petri-dishes onto gauze discs moistened with distilled water. There were two dishes of each species for each nutrient treatment. The number of seeds of each species was the same as in the previous NO_3-N/NH_4-N experiment. These dishes were kept at 20 °C for 5 days until all the seeds had germinated. The gauze discs were then transferred to the appropriate nutrient solutions in plastic basins. The rim of each disc was a thick ring of wax which supported the seedlings in the solutions. There were 16 nutrient treatments consisting of 4 levels of phosphorus, 4 levels of nitrate nitrogen and 9

different phosphorus/nitrogen combinations in the following concentrations (values are in mM or me./l):

Treatment	1	2	3	4
mM NO_3	0	1.6	8	32
me. NO_3	0	1.6	8	32
mM PO_4	0	0	0	0
me. PO_4	0	0	0	0

Treatment	5	6	7	8
mM NO_3	0	1.6	8	32
me. NO_3	0	1.6	8	32
mM PO_4	0.16	0.16	0.16	0.16
me. PO_4	0.48	0.48	0.48	0.48

Treatment	9	10	11	12
mM NO_3	0	1.6	8	32
me. NO_3	0	1.6	8	32
mM PO_4	0.8	0.8	0.8	0.8
me. PO_4	2.4	2.4	2.4	2.4

Treatment	13	14	15	16
mM NO_3	0	1.6	8	32
me. NO_3	0	1.6	8	32
mM PO_4	4.04	4.04	4.04	4.04
me. PO_4	12.12	12.12	12.12	12.12

The concentrations of other macronutrient ions were the same in each of the treatments (except for sulphate - see previous experiment) and were as follows:

ION	mM/l	me./l
K+	6	6
Ca++	4	8
Mg++	1	2
SO_4 --	variable	variable

Phosphorus was supplied in the form of KH_2PO_4, and nitrogen was supplied as $Ca(NO_3)_2 . H_2O$ and KNO_3. Any differences in the other ions due to various levels of P and N were compensated for by additional amounts of $CaSO_4 . 2H_2O$ or K_2SO_4. Each treatment was supplied with the following micronutrients:

Micronutrient	µM/l.	Micronutrient	µM/l.
KCl	100	$ZnSO_4 . 7H_2O$	4
H_3BO_3	50	$CuSO_4 . 5H_2O$	1
$MnSO_4 . H_2O$	4	H_2MoO_4	1
Fe-EDTA	40		

As was the case with the previous experiment, the pH of each solution was adjusted to 5.5. The pH was checked twice weekly and adjusted to 5.5 with either dilute $Ca(OH)_2$ or 0.02N H_2SO_4. The nutrient solutions were completely renewed every 2 weeks. The basins containing the different nutrient treatments were originally set out in the glasshouse in a random arrangement. They were rotated twice weekly. The growth of the seedlings in these various treatments continued for 8 weeks, after which they were harvested.

Harvesting and pre-treatment of tissue samples were carried out as previously described. The analysis of the soluble sugars of each species was done according to the method described at the beginning of this chapter. Also described there, is the method of electronic GLC peak measurement which was unique to this experiment.

Results

The data from this experiment was so massive that the clearest and most sensible presentation of it is in graphical form. In every case, each point which is connected by lines on these graphs is the mean of the 2 replicates of each species and treatment in this experiment. In order to keep these illustrations as clear as possible, the standard deviation of each point is not shown. Instead, each graph shows the maximum and minimum points in each treatment.

The first set of figures (3.10 a-d) shows the effect of changing nitrate-nitrogen on fructose concentration in the 4 species. There is a separate diagram for each phosphorus level. The results were arranged this way first of all to allow comparison with the nitrogen experiments. The third phosphorus level (2.4me./l) is closest to that used in the previous experiment.

Figure 3.8a shows that fructose concentration responds differently in A. tenuis and F. rubra than in D. glomerata and L. perenne when nitrate-nitrogen is increased in the absence of phosphorus. Analysis of variance showed that the variability due to treatments, species differences and species/treatment interaction at this phosphorus level was significant in each case at the 5% level. Furthermore, A. tenuis and F. rubra

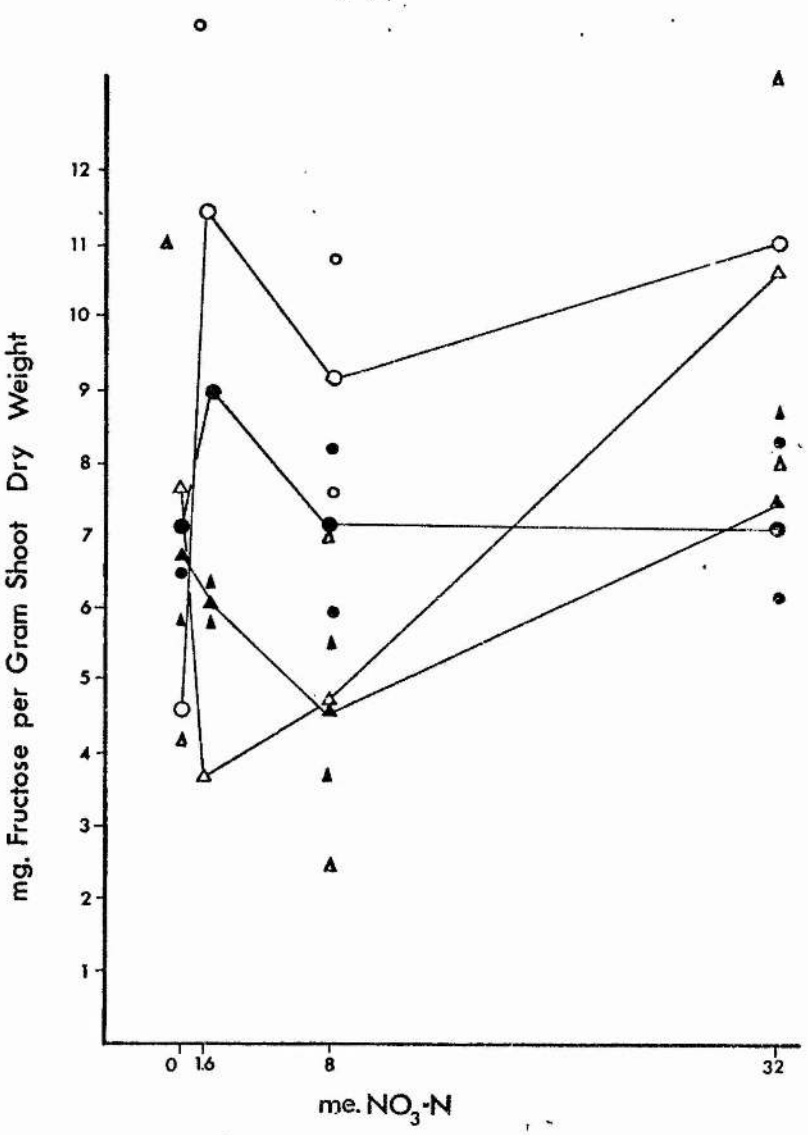


Figure 3.10a: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the fructose content of 4 species. The amount of phosphorus in each treatment was 0 me./l. The sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

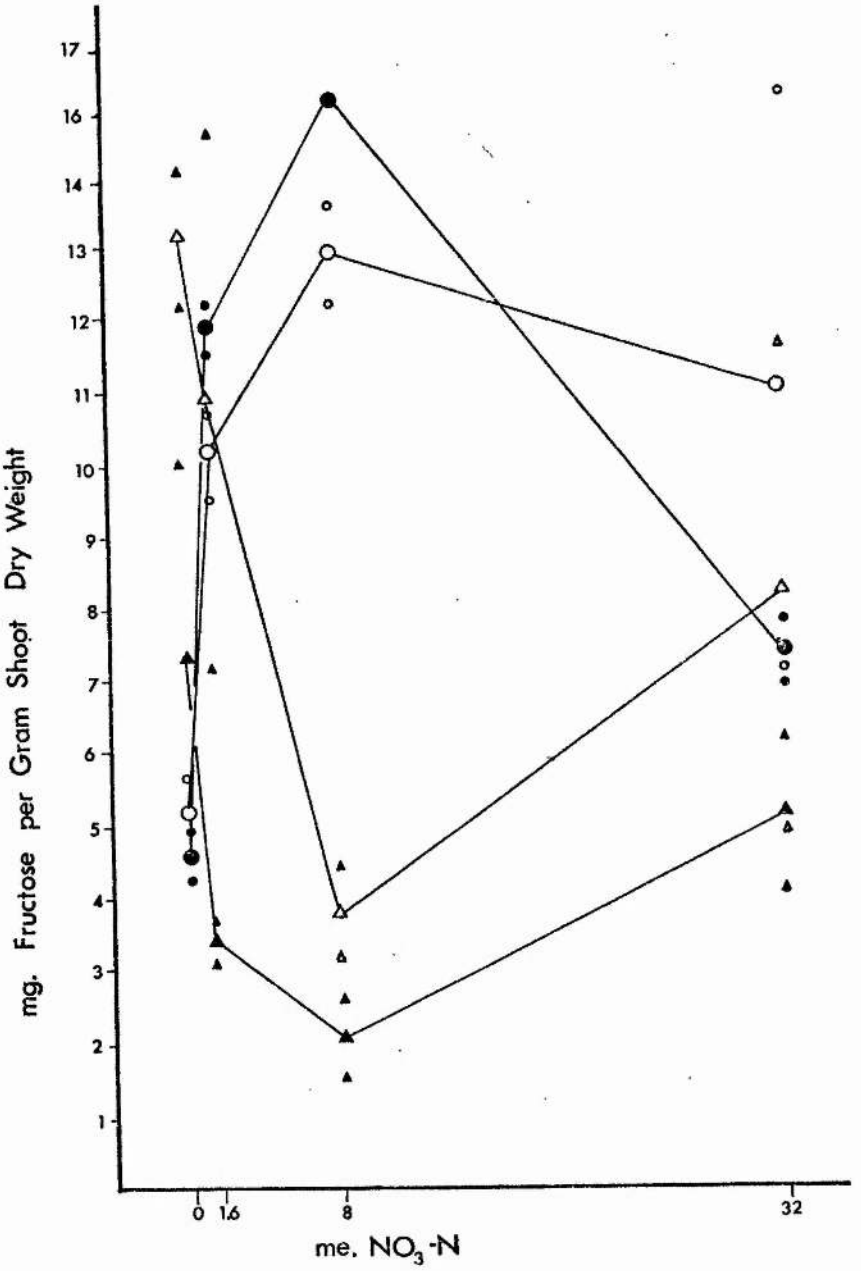


Figure 3.10b: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the fructose content of 4 species. The amount of phosphorus in each treatment was 0.48 me./l. The sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are shown, and the actual values of the replicates are shown.

○ = L. perenne ● = D. glomerata
▲ = A. tenuis △ = F. rubra

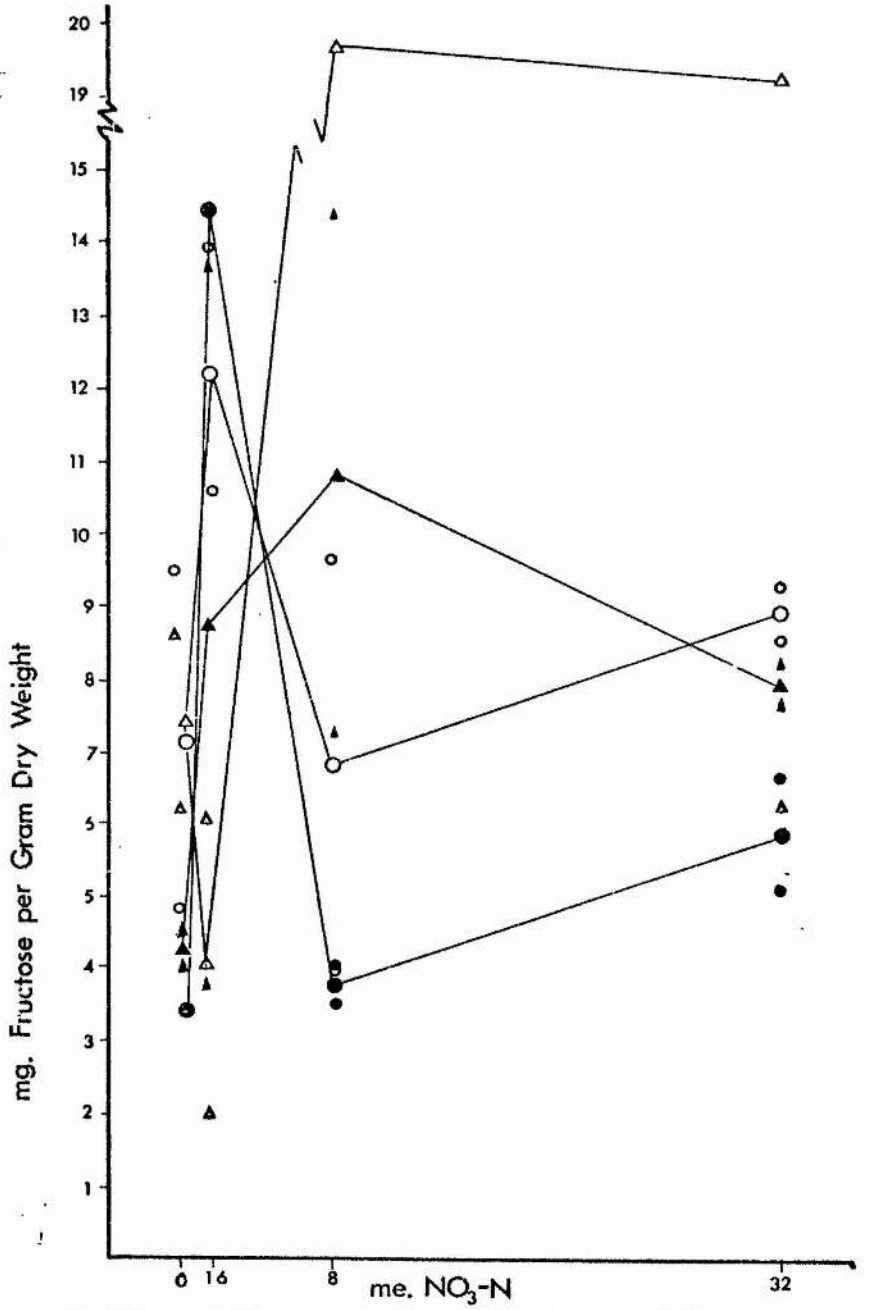


Figure 3.10c: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the fructose content of 4 species. The amount of phosphorus in each treatment was 2.4 me./l. The sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown (note change of scale).

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

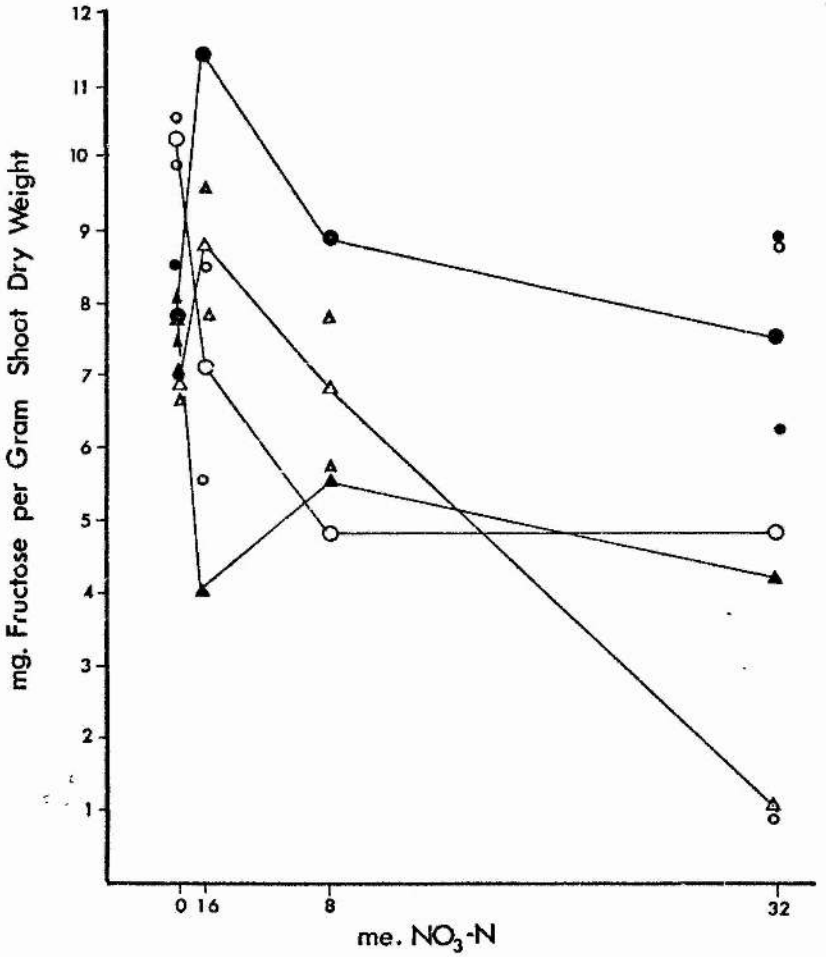


Figure 3.10d: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the fructose content of 4 species. The amount of phosphorus in each treatment was 12.12 me./l. The sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

were seen to be statistically different from D. glomerata and L. perenne. The first two species showed a marked decrease in fructose content as nitrate concentration was increased. When 1.6-8 me./l NO -N was reached, fructose began to increase rapidly as 32 me./l NO_3-N was approached. L. perenne and D. glomerata on the other hand, showed a marked increase in fructose as NO_3-N was increased from 0-1.6 me./l. This was followed by a decrease when up to 8 me./l NO_3-N was added. When the NO_3-N was increased further, fructose concentration increased slightly in L. perenne and levelled off in D. glomerata. Fructose response to the same NO_3-N levels when 0.48 me./l phosphorus was present, (Figure 3.10b) was basically the same as that seen in the previous figure. The variability due to species response was highly significant ($P<0.001$), and that due to interaction effects was significant at the 1% level. The reduction in sugar concentration in A. tenuis and F. rubra caused by the initial increase in NO_3-N was much greater when 0.48 me./l P was present, as was the increase in fructose concentration seen in L. perenne and D. glomerata. The reaction of these species to increasing NO_3-N at the next phosphorus level (2.4 me./l) was very different. F. rubra still responded to the first addition of nitrate with a decrease in fructose concentration, but this was followed by a

remarkable accumulation of sugar as nitrate was added. The other three species, including A. tenuis, responded to the first nitrate addition with an increase in fructose concentration. Fructose concentration in A. tenuis continued to rise until at least the 8me./l NO_3-N level was reached, and sugar concentration fell off as the 32 me./l level was reached. L. perenne and D. glomerata, however, showed a marked decrease in fructose between the 1.6 and 8 me./l NO_3-N levels. As 32 me./l NO_3-N was approached, fructose in these species tended to increase in concentration. Though these differences can be seen, they were not statistically different. When the highest phosphorus level was examined (12.12 me./l), the response of these species was again very different. L. perenne showed a continuous decrease in fructose concentration over the range of NO_3-N levels. This tended to level off between 8 and 32 me./l NO_3-N. A. tenuis was similar in its response. D. glomerata and F. rubra, however, both showed an increase in fructose concentration with a NO_3-N increase above 0-1.6 me./l. Sugar concentration in these species then decreased over the rest of the nitrate treatments, quite dramatically in the case of F. rubra.

In order to see fructose response to phosphorus concentration more clearly, the data had to be

presented in a different way. If 3-dimensional diagrams are constructed with phosphorus and nitrogen concentrations on two of the axes and sugar concentration on the other, the changes in fructose with phosphorus can be followed through increasing NO_3-N concentration. Although this method is used to present the data (there is a separate diagram for each species), analysis of variance could still be used to compare the species and phosphorus treatment variability at the separate nitrogen levels. Again, as with the previous analysis, it was necessary to use separate 2 factor analyses with replication for each nitrogen level as there may have been an interaction between phosphorus and nitrate. At the lowest nitrate level (0 me./l), the variabilty due to phosphorus treatments, species differences and the interaction of these two was highly significant in all three cases ($P<0.001$). The same was true at the 1.6 me./l NO_3-N level, though the statistical significance was slightly less ($P<0.05$). When the treatments contained 8 me./l N, the variability due to phosphorus treatments and that due to species/treatment interaction were highly significant ($P<0.001$), while that due to species difference alone was significant only to the 5% level. When the treatments contained 32 me./l nitrogen, the variability in fructose concentration could not be

ascribed to species, treatment or interaction effects.

The 3-dimensional diagrams (Figures 3.11 a-d) show the responses of each species clearly as a series of fructose response curves at each nitrogen level. In A. tenuis, except where nitrate was missing, fructose was seen to accumulate when phosphorus was deficient (ie. 0 me./l). The addition of even a very small amount of phosphorus (0.48 me./l) resulted in a substantial decrease in the concentration of this sugar. Additional phosphorus resulted in an accumulation of fructose. As 12 me./l phosphorus was approached, fructose concentration again decreased. This pattern was true of all nitrogen levels except the 0 me./l NO_3-N treatments where exactly the opposite pattern was demonstrated. Maximum fructose concentration occurred at 2.4 me./l P and 8 me./l N. The minimum amount of sugar occurred in plants from treatments containing 0.48 me./l P and 8 me./l N.

Festuca rubra responded to increasing phosphorus in much the same way as did A. tenuis, but the amounts of fructose in the tissues were much greater. Additionally, fructose at both the 0 and 1.6 me./l nitrogen treatments showed initial accumulation, whereas in A. tenuis, this was only seen at the 0 me./l nitrogen treatment. At the 8 and 32 me./l NO_3-N levels the response of fructose to increasing phosphorus was

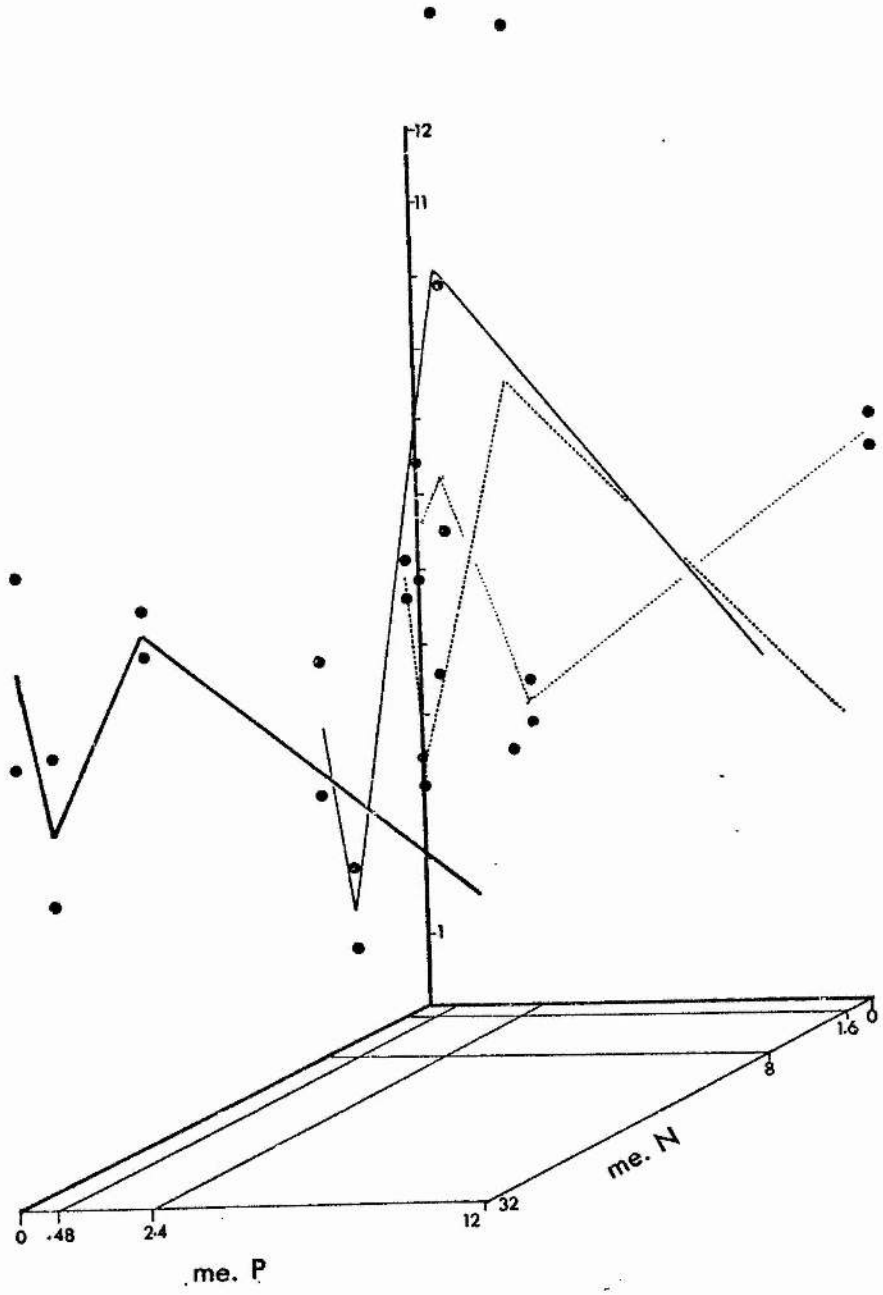


Figure 3.11a: 3-dimensional diagram illustrating the effect on fructose concentration of different amounts of phosphorus at 4 levels of NO_3-N in A. tenuis. The vertical axis is sugar concentration measured in mg fructose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

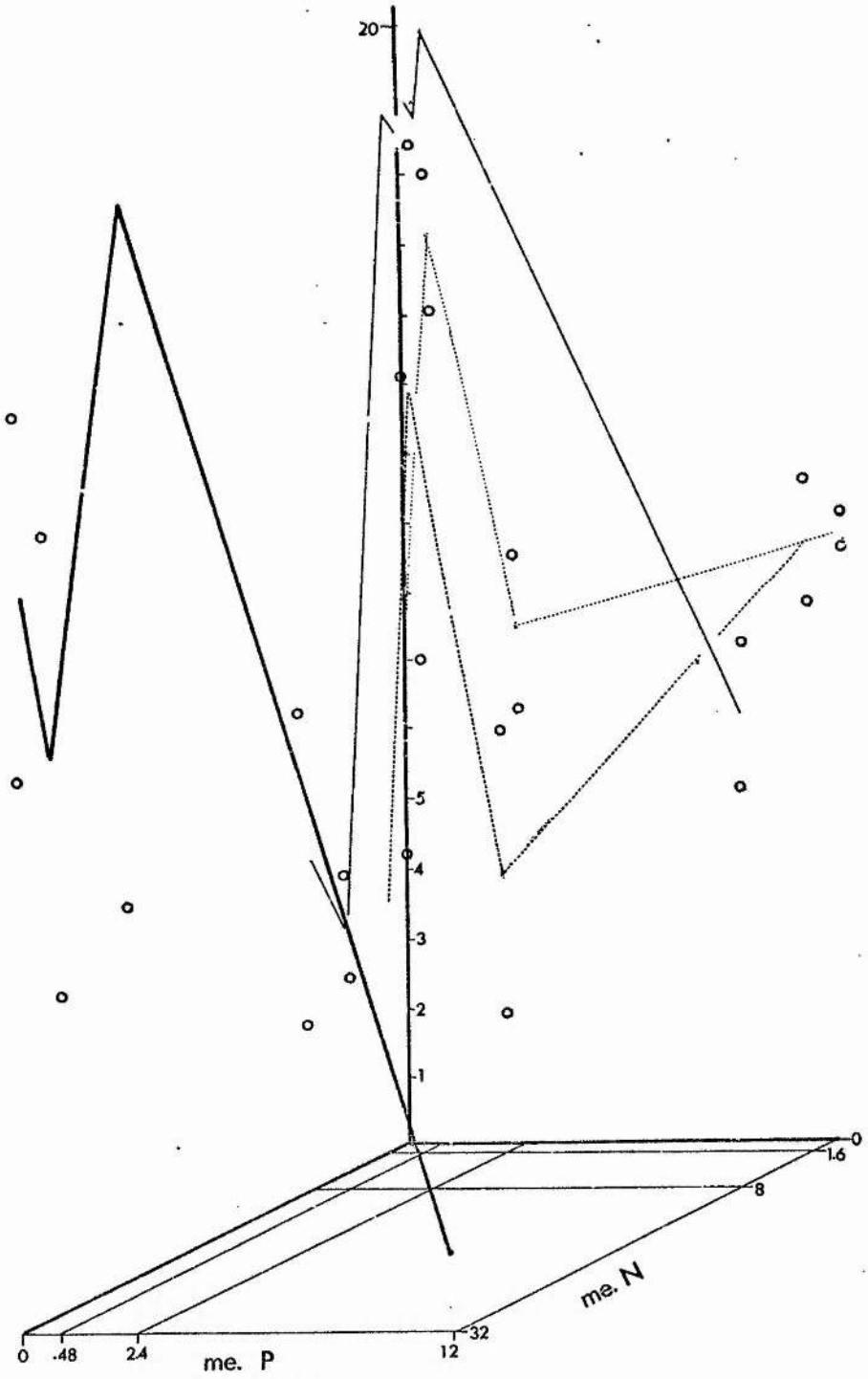


Figure 3.11b: 3-dimensional diagram illustrating the effect on fructose concentration of different amounts of phosphorus at 4 levels of NO_3-N in F. rubra. The vertical axis is sugar concentration measured in terms of mg fructose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown (note change of scale).

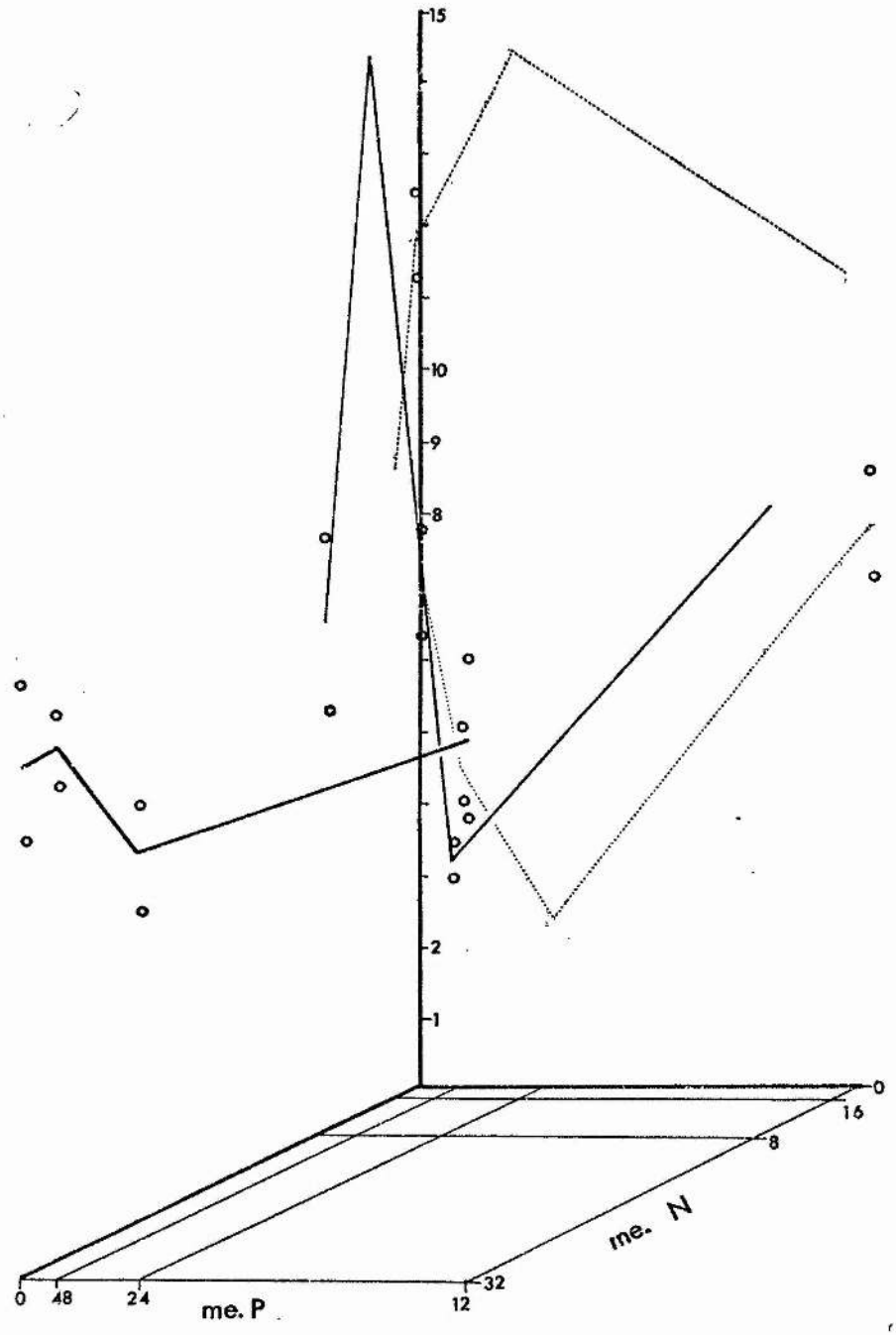


Figure 3.11c: 3-dimensional diagram illustrating the effect on fructose concentration of different amounts of phosphorus at 4 levels of NO_3-N in D. glomerata. The vertical axis is sugar concentration measured in mg fructose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

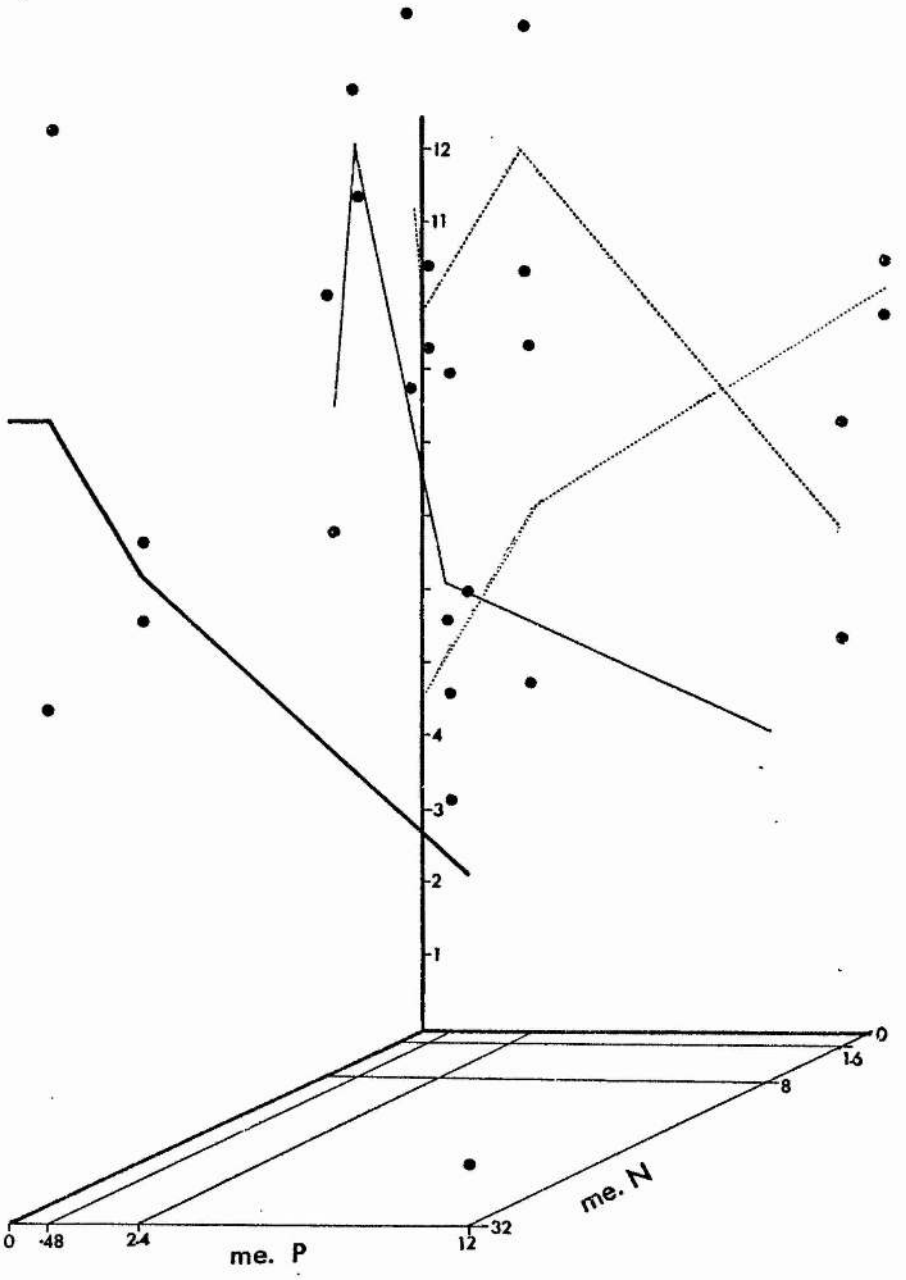


Figure 3.11d: 3-dimensional diagram illustrating the effect on fructose concentration of different amounts of phosphorus at 4 levels of NO_3-N in L. perenne. The vertical axis is sugar concentration measured in mg fructose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

much the same as that seen in A. tenuis at these levels. The concentrations were much greater than in A. tenuis, however, and the response curve of fructose at the highest nitrogen concentration showed a remarkable decrease in sugar concentration as the 12 me./l P level was approached. This was the lowest concentration of this sugar seen in any of the treatments. The greatest concentration of fructose in F. rubra was found in the treatment containing 2.4 me./l P and 8 me./l NO_3-N.

In both D. glomerata and L. perenne, the response of fructose concentration to phosphorus level varied much more, in both pattern and amount of sugar, according to nitrogen level than did either of the other species. When no nitrogen was present, the fructose response curve of D. glomerata showed a gradual decrease until 2.4 me./l P was reached. This was followed by a rapid accumulation of sugar as the 12 me./l P level was approached. When the series of treatments containing 1.6 me./l NO_3-N was examined, a pattern was seen which was the complete opposite of the 0 me./l NO_3-N treatments. The 8 and 32 me./l series of nitrogen levels exhibited fructose response curves which were typical of the lowest nitrate series of A. tenuis and F. rubra. That is, the sugar showed an initial increase in concentration from 0 to 0.48 me./l P followed by a decrease at the 2.4 me./l P treatment

and, lastly, an increase as the highest phosphorus level was approached. The greatest concentration of fructose occurred in the treatment containing 2.4 me./l P and 8 me./l N. The lowest concentration was brought about by a treatment containing 2.4 me./l P and 0 me./l N.

In the 0 me./l nitrogen series, L. perenne showed a gradual, continuous increase in fructose concentration with increasing phosphorus. This pattern was reversed in the 1.6 me./l nitrogen series, with the response curve typical of the higher nitrate series in A. tenuis and F. rubra. In the 8 me./l nitrogen series, the pattern was reversed again. This time fructose concentration increased with increasing phosphorus until the 0.48 me./l level was reached. Thereafter, the sugar decreased over the remaining range of phosphorus levels. The last response curve, that in which nitrate-nitrogen was present in the amount of 32 me./l, showed simply a gradual decrease in fructose as phosphorus was increased.

When the response of the other reducing-sugar, glucose, to nitrogen was examined (Figures 3.12 a-d) at each separate phosphate level, some of the patterns were seen to be quite similar to those of fructose. In the 0 me./l P level, however, the variability due to species difference was not statistically significant.

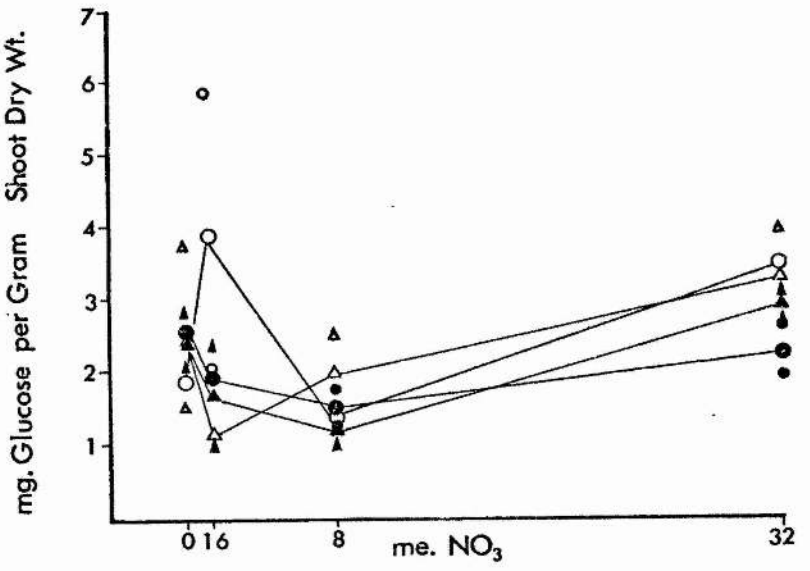


Figure 3.12a: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the glucose content of 4 species. The amount of phosphorus in each treatment was 0 me./l. The sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

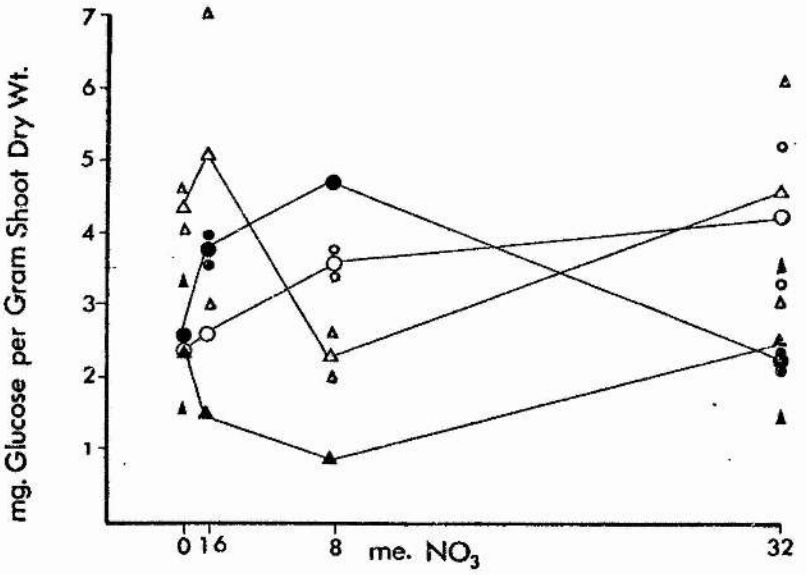


Figure 3.12b: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the glucose content of 4 species. The amount of phosphorus in each treatment was 0.48 me./l. The sugar content was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

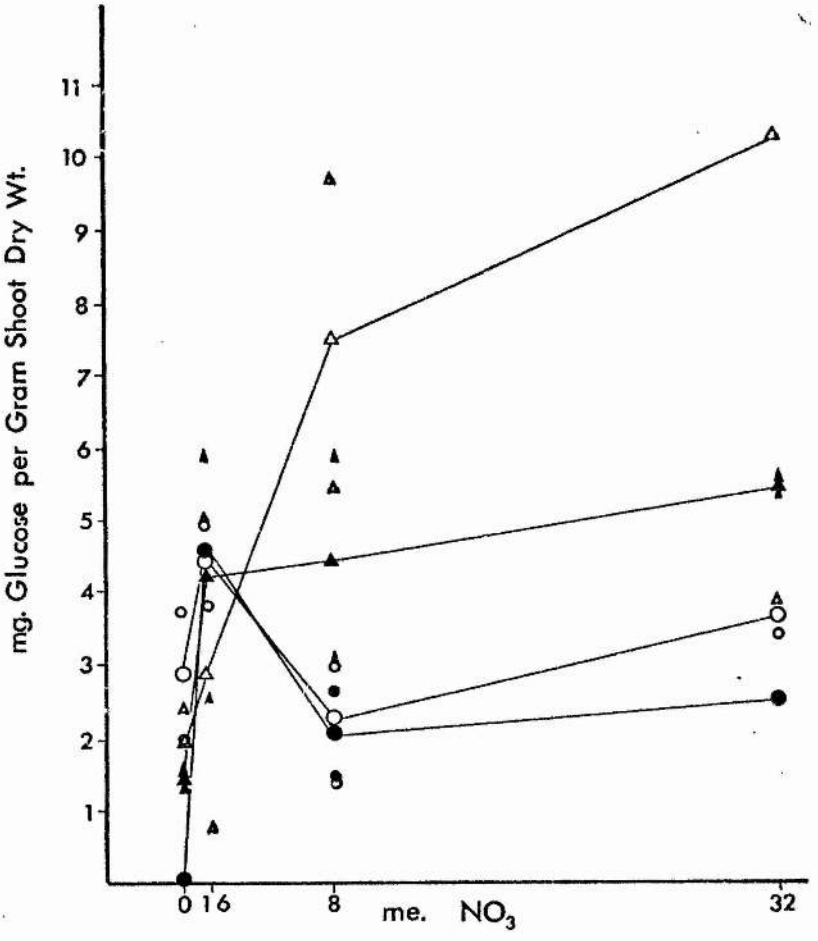


Figure 3.12c: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the glucose content of 4 species. The amount of phosphorus in each treatment was 2.4 me./l. The sugar was measured in terms of mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

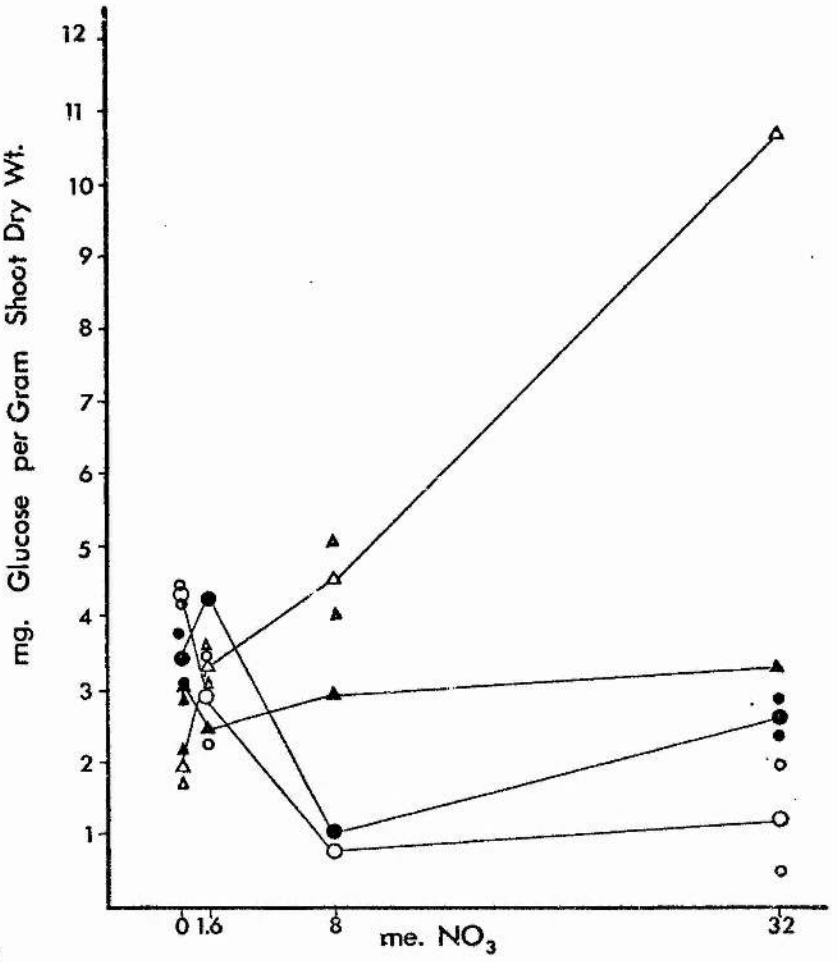


Figure 3.12d: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the glucose content of 4 species. The amount of phosphorus in each treatment was 12.12 me./l. The sugar content was measured in terms on mg sugar per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

In general, the response of glucose at this level of phosphorus was one of initial decrease, indicating accumulation of sugar during conditions of nitrogen deficiency, followed by a gradual accumulation as nitrogen was increased. L. perenne was the exception in that it showed an initial increase in sugar. The glucose response curves at the 0.48 me./l phosphorus level showed the same patterns as those of fructose at the same level, although the amounts of fructose at each nitrogen level were much greater. In general, L. perenne and D. glomerata showed an increase in glucose concentration at low nitrogen levels followed by a decrease at higher ones. In A. tenuis and F. rubra, there was generally a decrease at low levels of nitrogen and a gradual increase as nitrogen fertilisation was increased. The variability due to species differences was significant at the 1% level. The third phosphorus level (2.4 me./l P) resulted in a series of response curves of the same pattern as those of fructose at the same level. Glucose in A. tenuis and F. rubra tended to accumulate as nitrogen level was increased. D. glomerata and L. perenne showed an initial increase in glucose, but this was followed by a decrease in concentration of sugar as the 8 me./l level was approached, bringing the total glucose concentration of these species well below that of A.

tenuis and F. rubra. As the nitrogen concentration was further increased, D. glomerata and L. perenne showed a gradual glucose accumulation. When each treatment contained 12.12 me./l P, glucose showed nearly the same pattern of response in L. perenne as fructose at this level, that is, a gradual decrease in concentration as nitrogen is increased. The actual concentrations of fructose were much greater, however. D. glomerata showed a pattern of initial glucose accumulation from 0 to 1.6 me./l N, a period of decreasing glucose with increasing nitrogen to the 8 me./l N level, and a final phase of gradual glucose accumulation as nitrogen was increased from 8 to 32 me./l. A. tenuis, though this species showed an initial, small decrease in glucose concentration, maintained a fairly constant sugar concentration over the range of nitrogen levels. F. rubra was remarkable in that glucose was accumulated rather dramatically over the range 0-32 me./l nitrogen (the fructose response curve at this level of phosphorus showed a dramatic decrease in sugar concentration). The analysis of this series of response curves showed that the variability due to treatments, species differences and interaction effects was highly significant ($P<0.001$) in all three cases.

Figures 3.13 a-d show that the glucose response curves illustrating the effect of phosphorus on glucose

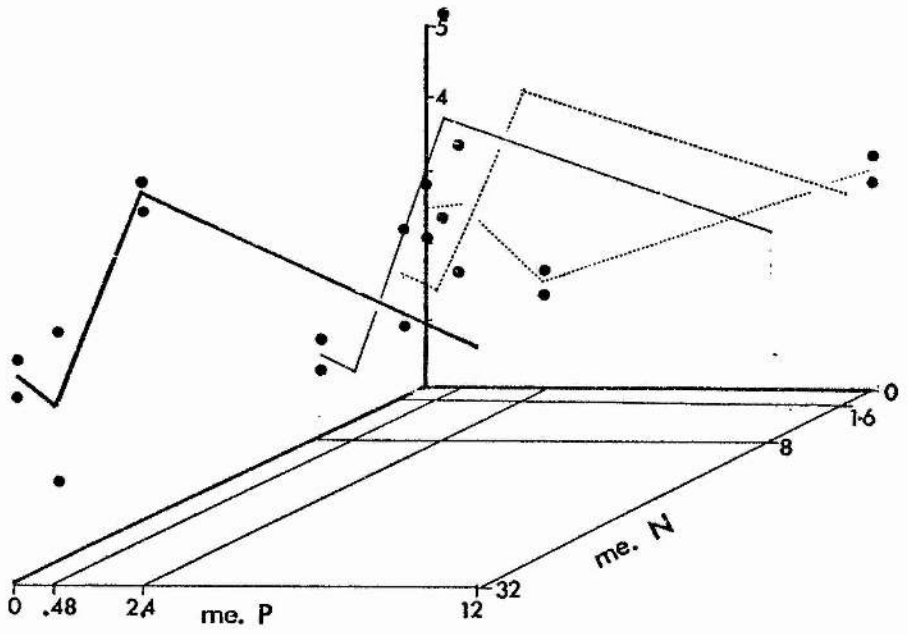


Figure 3.13a: 3-dimensional diagram illustrating the effect on glucose concentration of different amounts of phosphorus at 4 levels of NO_3-N in A. tenuis. The vertical axis is sugar concentration measured in mg glucose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

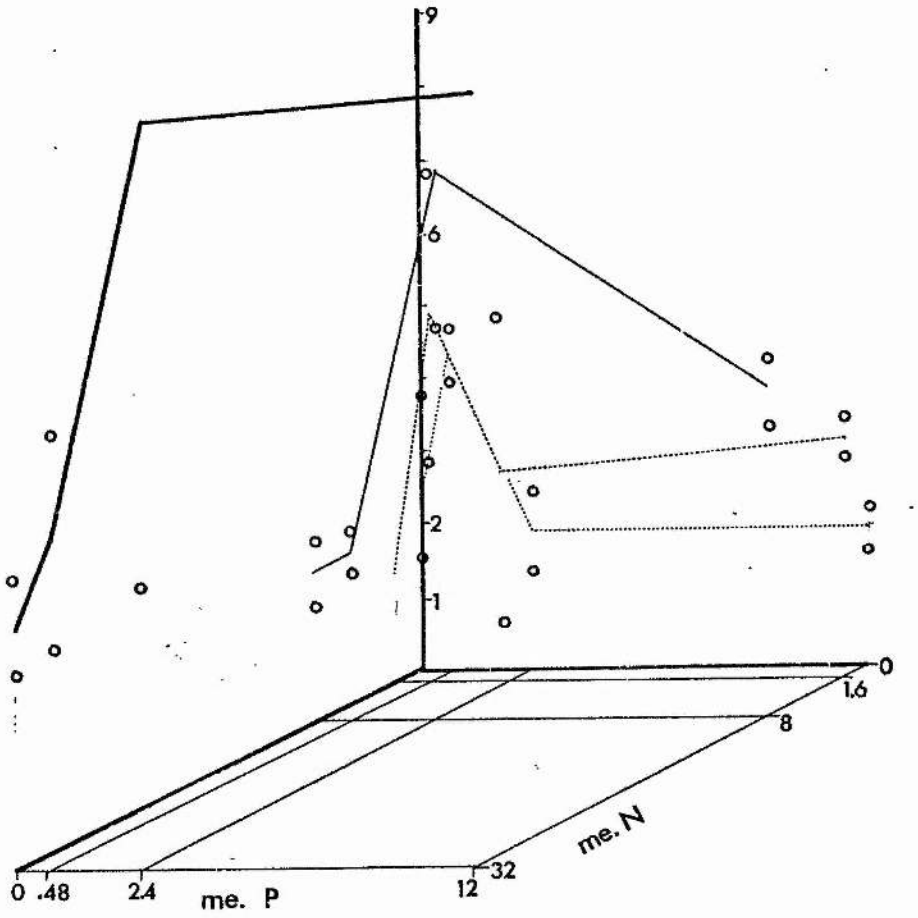


Figure 3.13b: 3- dimensional diagram illustrating the effect on glucose concentration of different amounts of phosphorus at 4 levels of NO_3-N in F. rubra. The vertical axis is sugar concentration measured in mg glucose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

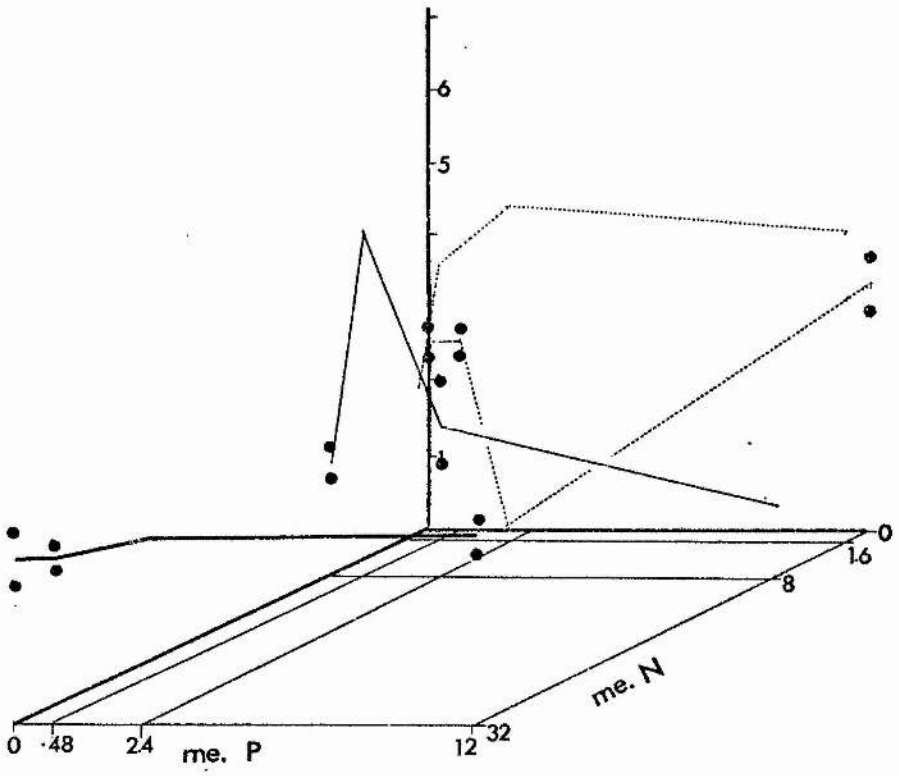


Figure 3.13c: 3-dimensional diagram illustrating the effect on glucose concentration of different amounts of phosphorus at 4 levels of NO_3-N in D. glomerata. The vertical axis is sugar concentration measured in mg glucose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

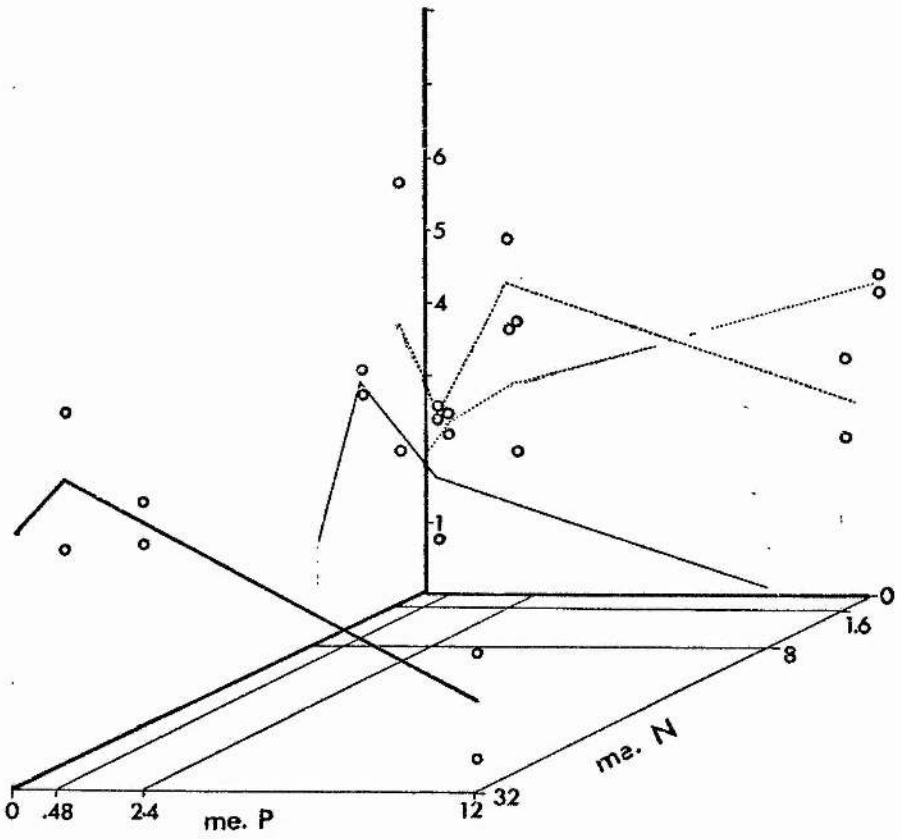


Figure 3.13d: 3-dimensional diagram illustrating the effect on glucose concentration of different amounts of phosphorus at 4 levels of NO_3-N in L. perenne. The vertical axis is sugar concentration measured in mg glucose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

concentration bear a close resemblance to figures 3.11 a-d which illustrated the fructose response curves. The patterns of those response curves in A. tenuis were the same patterns exhibited by fructose. The only difference being the greater concentrations of fructose accumulated at each level and the greater uniformity of the glucose response: with the exception of the series where nitrogen was lacking, the amount of nitrogen had only a slight effect on the glucose response curve. The same was generally true of F. rubra: the concentrations of glucose tended to be lower than those of the equivalent fructose response curves, the equivalent response curves of fructose and glucose were similar, and nitrogen level apparently had less effect on the response curves of glucose than on those of fructose. The exception to this was the response curve of glucose at the highest nitrogen level. Whereas the response curve of fructose showed a great sugar accumulation followed by a dramatic decrease in sugar concentration as phosphorus level increased, the glucose response curve was one of dramatic accumulation of glucose followed by a plateau as the 12.12 me./l P level was approached.

The response curves of the remaining species, L. perenne and D. glomerata (figures 3.13 c-d), like those of A. tenuis and F. rubra, showed patterns remarkably

like those of the fructose response curves. Indeed, the only real differences were the degree of response to phosphorus (the glucose concentrations were much less than those of fructose) and the degree of response curve reaction to nitrogen level (eg. phosphorus/nitrogen interaction).

If an analysis of variance is applied to the data such that the two factors are species and phosphorus level, and this is repeated for each nitrogen level separately, the following results are found: the variability due to treatment effects was significant for the first three nitrogen levels (0, 1.6, and 8 me./l) with $P<0.001$, $P<0.05$ and $P<0.001$ respectively. The variability due to species differences was only significant at the 8 and 32 me./l nitrogen levels ($P<0.01$ for both). There was a significant interaction effect between species and treatment for the 0 me./l ($P<0.001$) and the 8 me./l nitrogen level ($P<0.001$).

The remaining sugar to be studied in detail is the non-reducing sugar sucrose. The response of sucrose concentration to increasing nitrogen, examined at each phosphorus level, is illustrated in figures 3.14 a-d. The response curves of the four species in the 0 me./l P series were very similar in pattern and response. With increasing nitrogen, sucrose accumulated to a maximum between 1.6 me./l and 8 me./l nitrogen, after

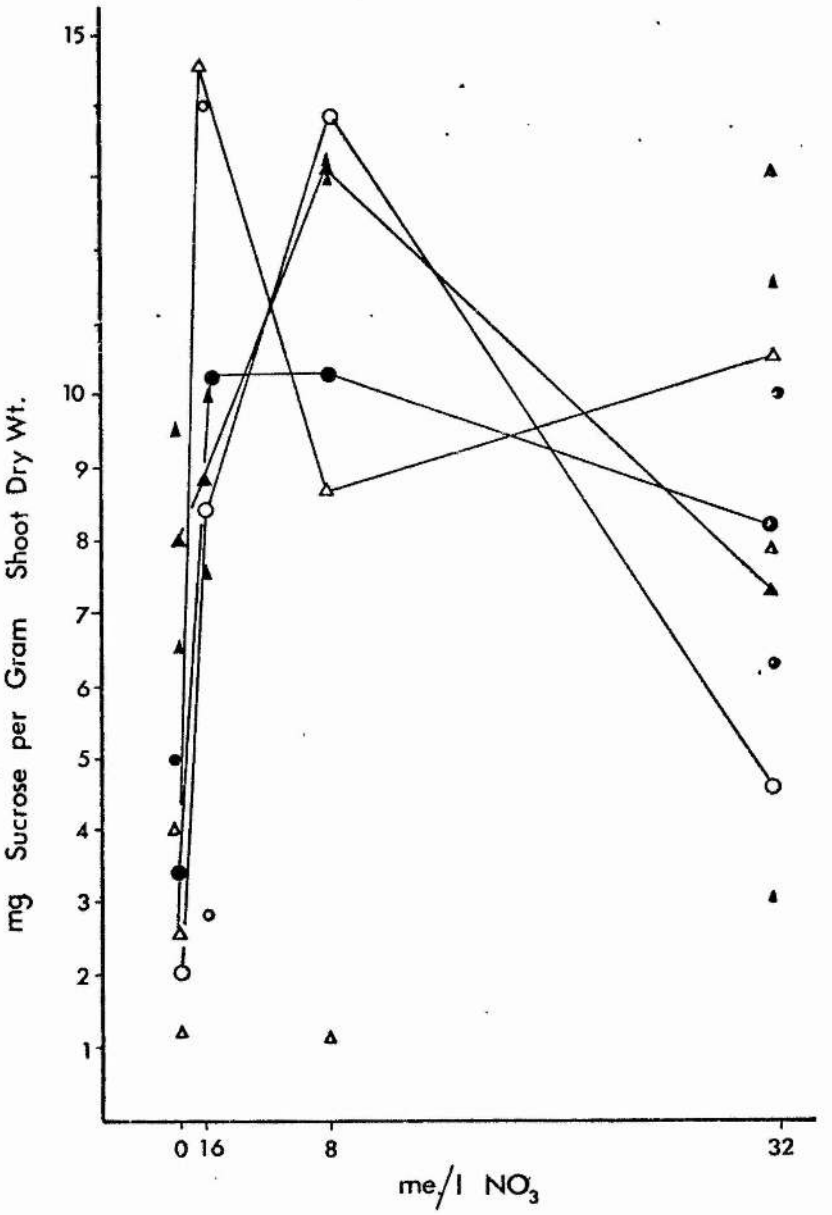


Figure 3.14a: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the sucrose content of 4 species. The amount of phosphorus in each treatment was 0 me./l. The sugar was measured in terms of mg sucrose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○ = L. perenne ● = D. glomerata
▲ = A. tenuis △ = F. rubra

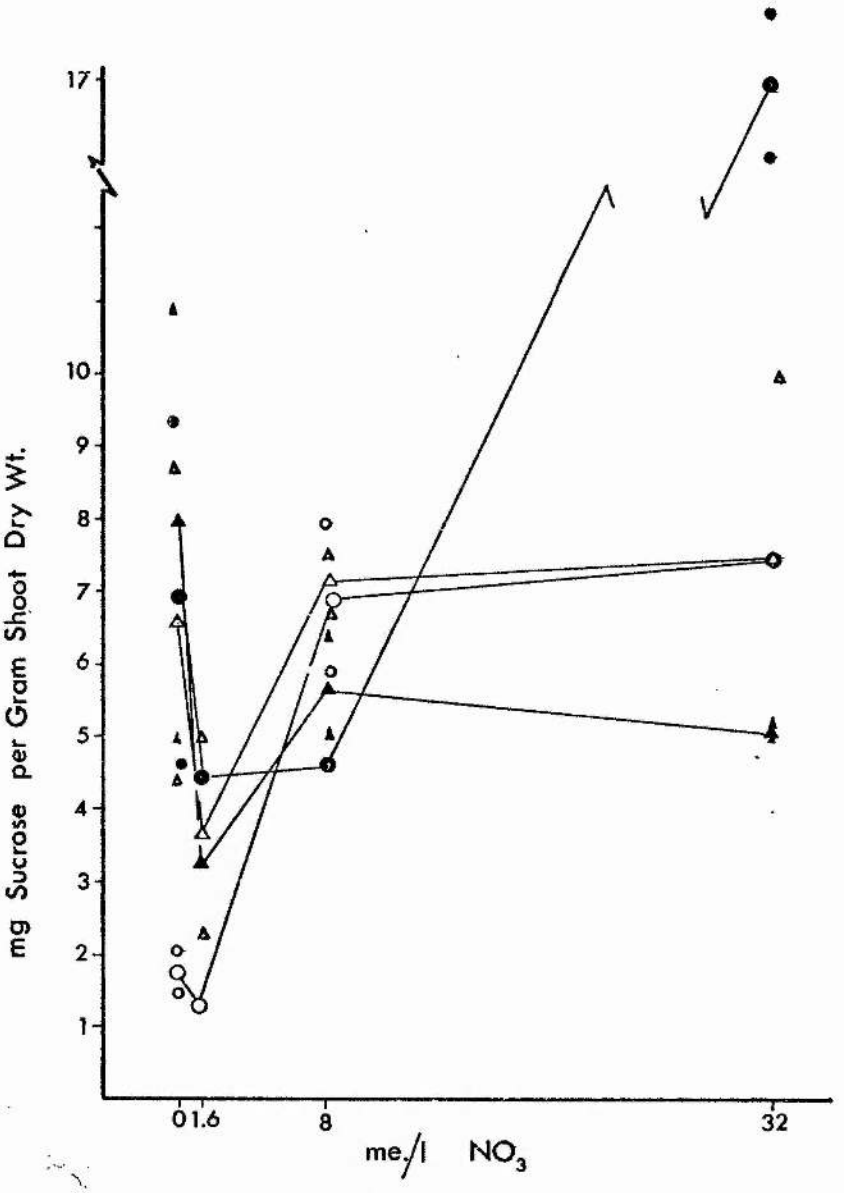


Figure 3.14b: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the sucrose content of 4 species. The amount of phosphorus in each treatment was 0.48 me./l. The sugar was measured in terms of mg sucrose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

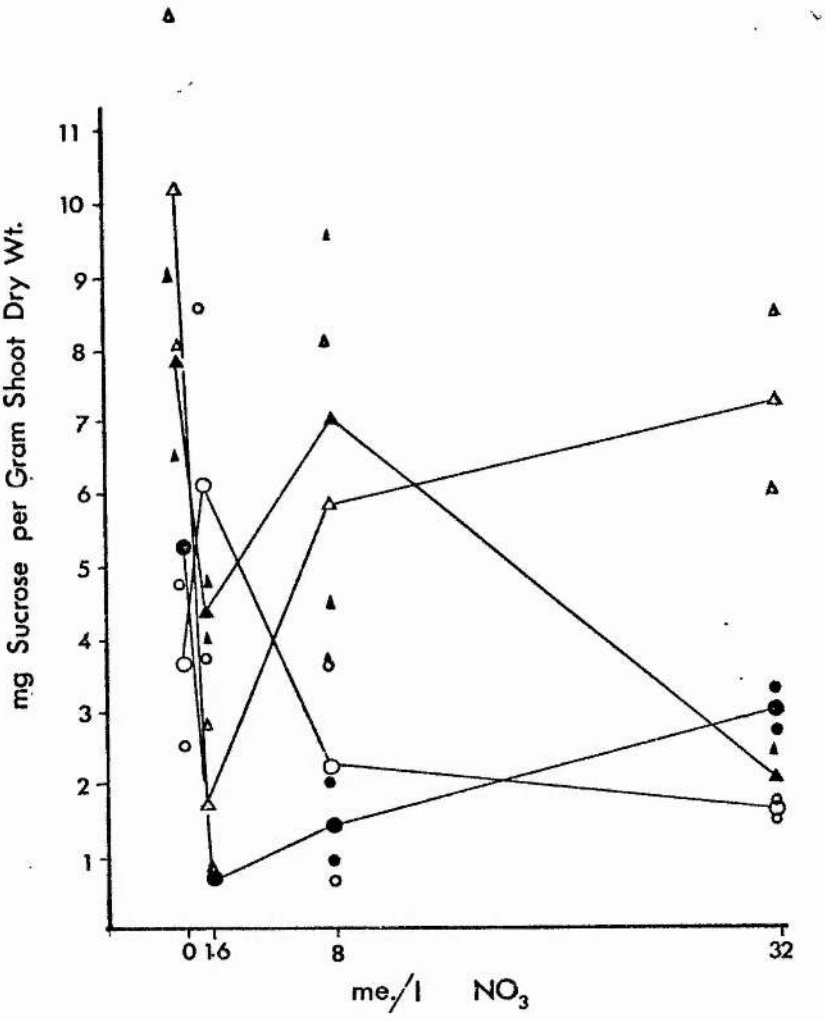


Figure 3.14c: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the sucrose content of 4 species. The amount of phosphorus in each treatment was 2.4 me./l. The sugar was measured in terms of mg sucrose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○ = L. perenne ● = D. glomerata
▲ = A. tenuis △ = F. rubra

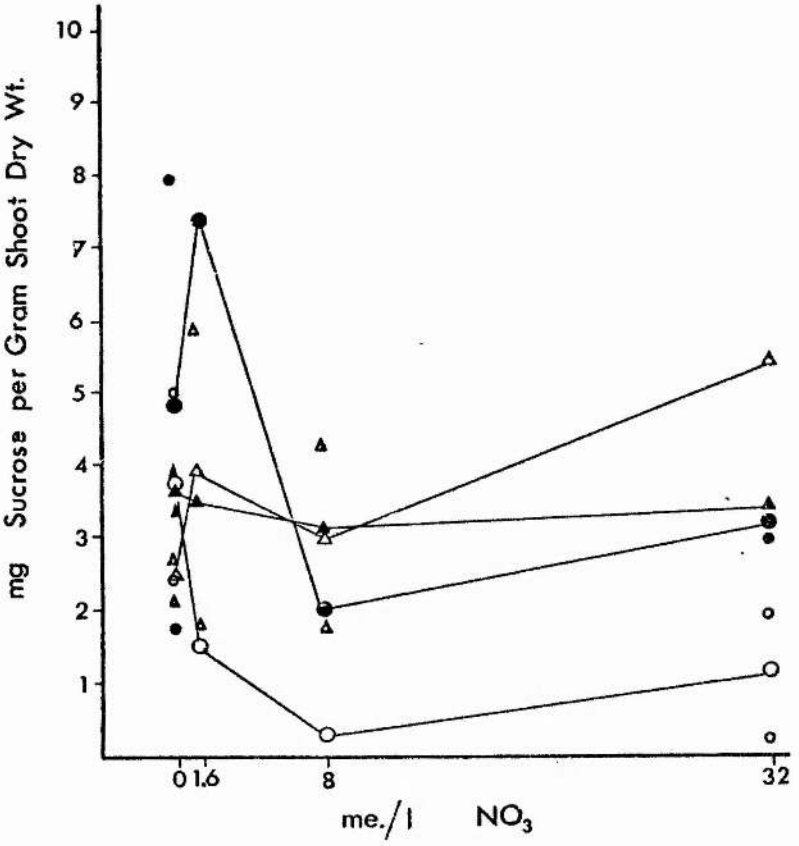


Figure 3.14d: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the sucrose content of 4 species. The amount of phosphorus in each treatment was 12.12 me./l. The sugar was measured in terms of mg sucrose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

which any further increase in nitrate nitrogen resulted in a decrease of sucrose concentration. F. rubra was slightly different in that the maximum sugar concentration occurred at a lower nitrogen level (1.6 me./l) than the other species, and the sugar decrease from 1.6 to 8 me./l N was followed by a gradual accumulation of sucrose. When 0.48 me./l phosphorus was included in an identical series of nitrogen treatments, the initial response of all four species to added nitrogen was a decrease in sugar concentration. This was the opposite response to the case where no phosphorus was present. Plants of all four species then showed a higher sucrose concentration when grown in the 8 me./l N treatments than in the 1.6 me./l N treatments. D. glomerata, however, showed only a slight sucrose increase at this stage, and this was followed by a rapid accumulation of sucrose as the 32 me./l level was approached. The other species showed very little difference in sucrose concentration between the 8 and 32 me./l nitrogen treatments at this phosphorus level. Analysis of variance revealed that there were significant differences between nitrogen treatments ($P<0.001$), species ($P<0.05$) and species/treatment interactions ($P<0.01$). Although significant interaction effects usually indicate that the species respond to treatments in different ways, examination of figure

3.14b shows that this significance was probably due to the great differences in concentration of sugar in D. glomerata. The response curves are, with this exception, very similar.

When phosphorus was increased to 2.4 me./l, analysis of variance again showed that there was a significant difference between the species ($P<0.001$), and there was significant species/treatment interaction ($P<0.05$). In this series of response curves, the reaction of sucrose concentration in F. rubra was not very different from that seen in response to the 0.48 me./l P series. The only real difference was that the sucrose accumulated to a much greater concentration in F. rubra at deficient nitrogen levels (ie. 0 me./l) when 2.4 me./l P was present than when the treatments contained only 0.48 me./l P. The response curve of A. tenuis at this phosphorus level was also very similar to that of its response curve at the lower phosphorus level. The only difference here was that between 8 and 32 me./l N sucrose concentration decreased markedly instead of very slowly. L. perenne responded to nitrogen very differently at this higher phosphorus level. This species showed an initial increase in sucrose concentration to a maximum at the 1.6 me./l N level. After this maxima, any further increase in nitrogen concentration was accompanied by a decrease in

sucrose concentration. Like A. tenuis and F. rubra, D. glomerata showed an initial decrease in sucrose with increasing nitrogen, but after 1.6 me./l N was reached, sucrose tended to accumulate.

The species were also significantly different ($P<0.05$) in their responses to nitrogen at the highest phosphorus level, 12.12 me./l P. L. perenne and A. tenuis showed the same pattern of decrease in sucrose concentration with increasing nitrate-nitrogen. In L. perenne, sucrose decreased in concentration to a nitrogen level of 8 me./l, but futher increase in NO_3-N resulted in an accumulation of this sugar. A. tenuis, though it showed the same pattern of response, did not show marked changes in sucrose concentration as 8 me./l N was approached. Then sucrose accumulated as nitrogen concentration was increased.

As was the case with the other sugars, sucrose/phosphorus response curves were plotted for each species. This set of 3-dimensional diagrams is presented in figures 3.15 a-d. Under conditions of phosphorus deficiency (eg. 0 me./l P), A. tenuis accumulated sucrose in its tissues. When no nitrogen was present, there was a gradual decrease in sugar concentration as phosphorus was increased to the 2.4 me./l level. As phosphorus was increased beyond this concentration, there was a rapid decrease in sugar per

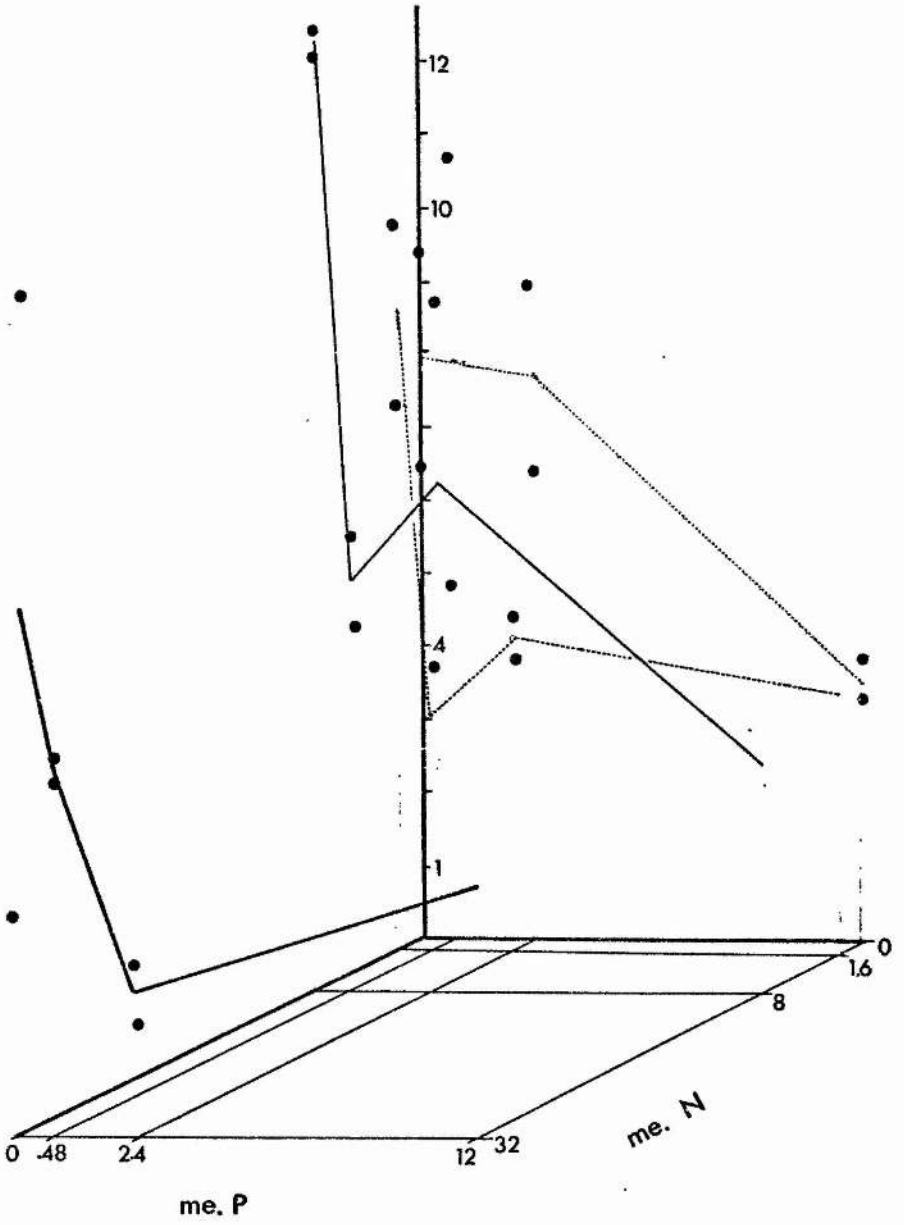


Figure 3.15a: 3-dimensional diagram illustrating the effect on sucrose concentration of different amounts of phosphorus at 4 levels of NO_3-N in A. tenuis. The vertical axis is sugar concentration measured in mg sucrose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

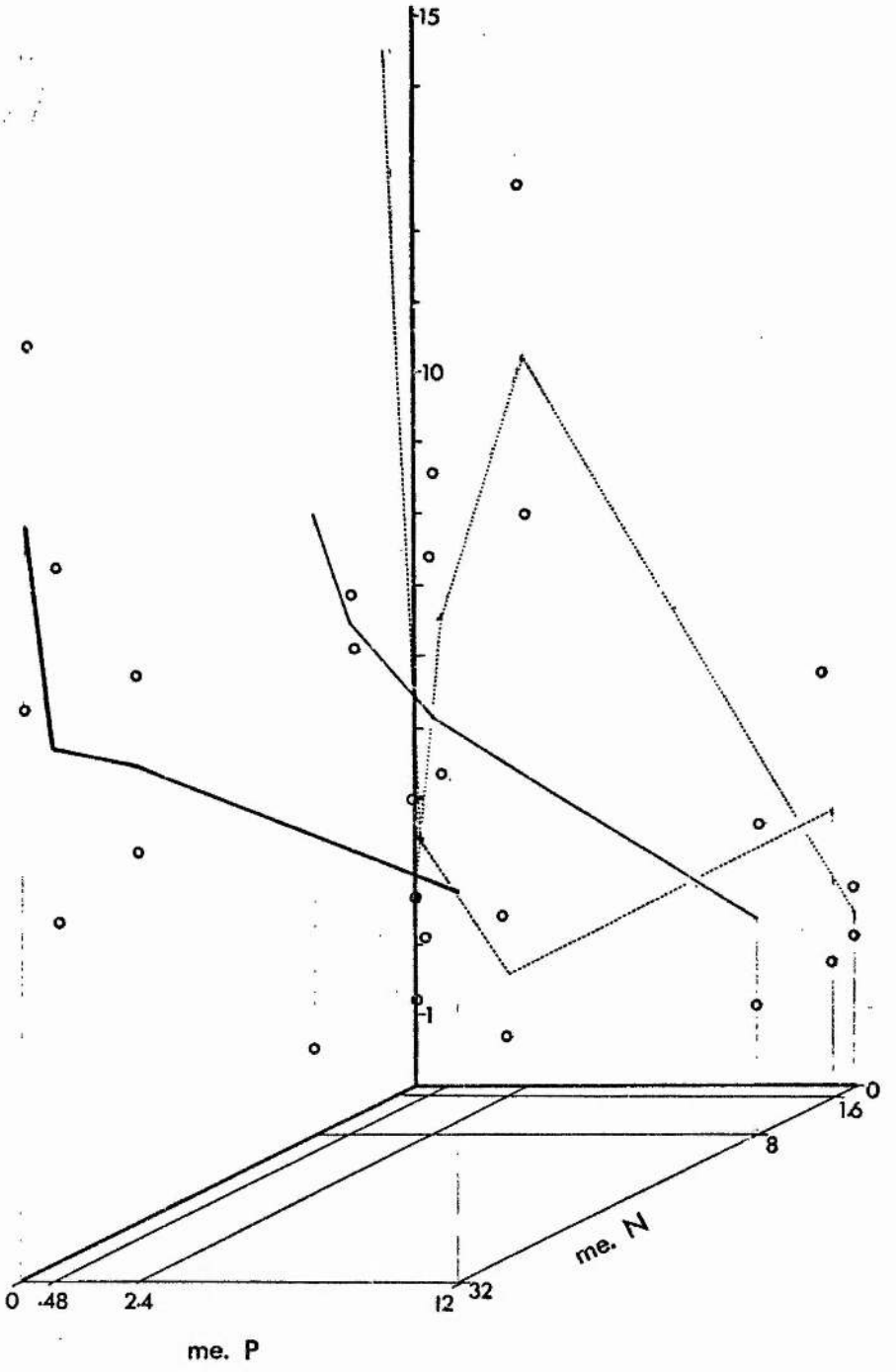


Figure 3.15b: 3-dimensional diagram illustrating the effect on sucrose concentration of different amounts of phosphorus at 4 levels of NO_3-N in F. rubra. The vertical axis is sugar concentration measured in mg sucrose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

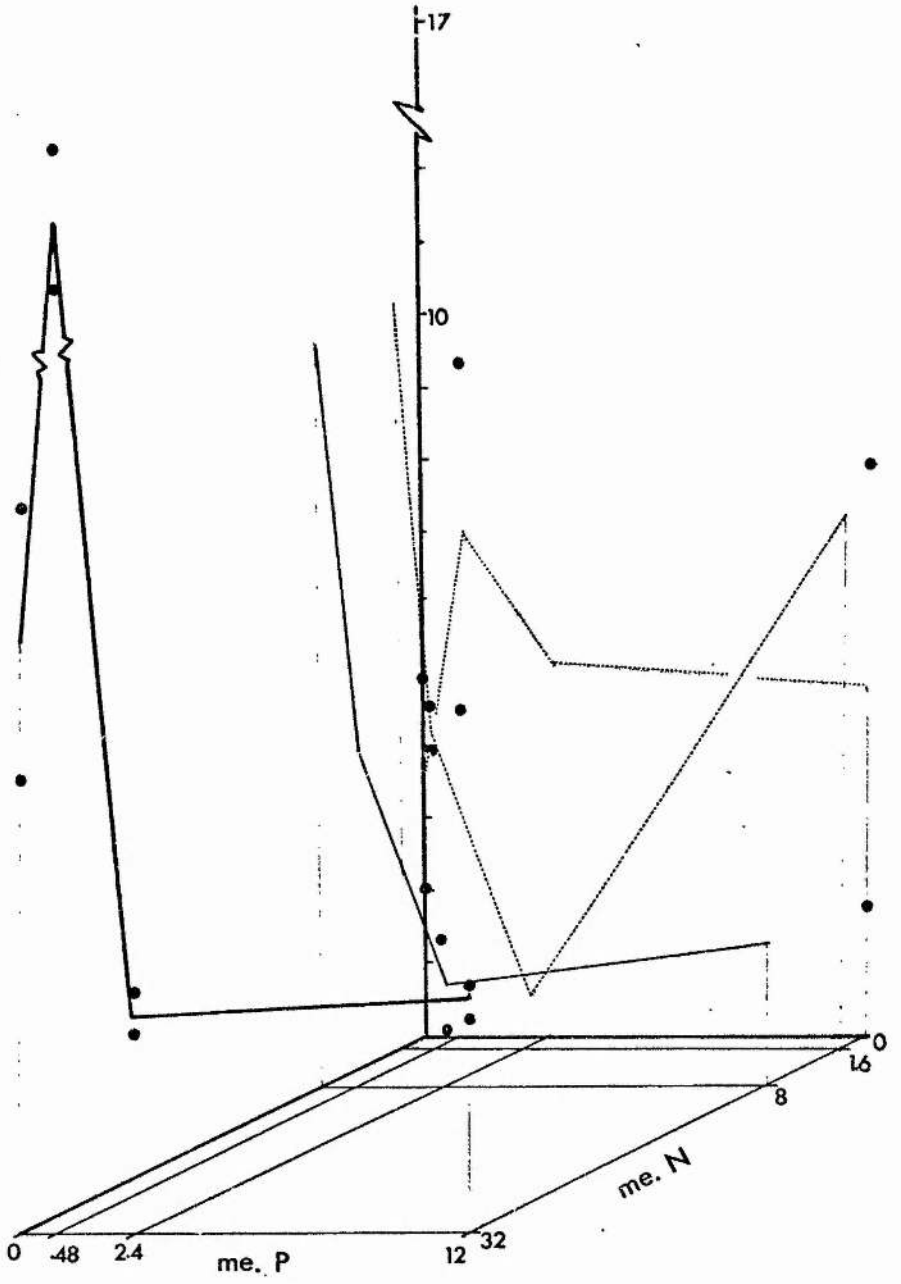


Figure 3.15c: 3-dimensional diagram illustrating the effect on sucrose concentration of different amounts of phosphorus at 4 levels of NO_3-N in D. glomerata. The vertical axis is sugar concentration measured in mg sucrose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

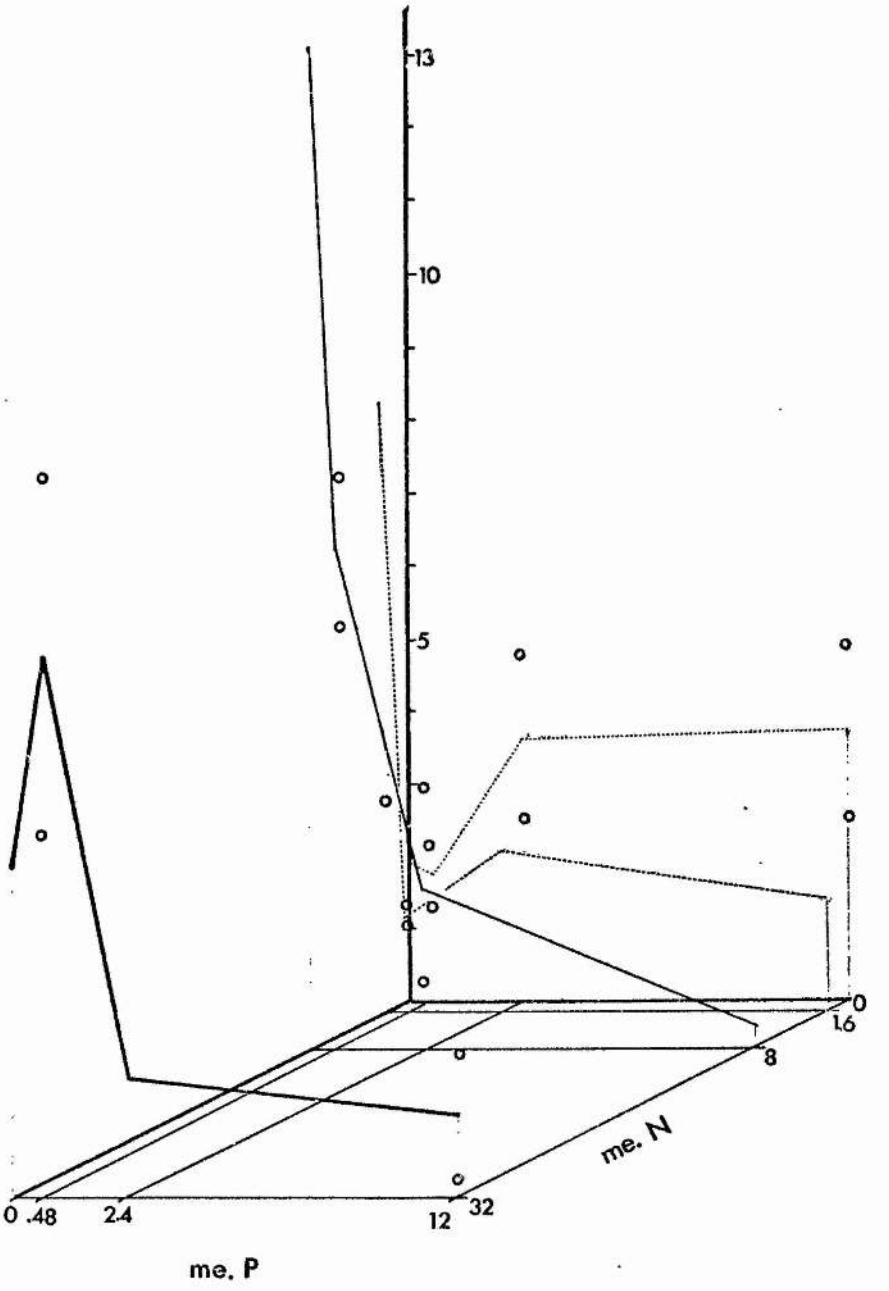


Figure 3.15d: 3-dimensional diagram illustrating the effect on sucrose concentration of different amounts of phosphorus at 4 levels of NO_3-N in L. perenne. The vertical axis is sugar concentration measured im mg sucrose per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

gram shoot dry weight. In the series of treatments containing 1.6 me./l N, the major change was the initial, rapid decrease in sugar concentration. This was seen at the next nitrogen level as well (8me./l). At the highest nitrogen level (32 me./l), the response was one of rapid decrease in sucrose up to 2.4 me./l P. Further increase in phosphorus was accompanied by an accumulation of sugar. The F. rubra responses were somewhat similar. When no nitrogen was present, sucrose accumulated until 2.4 me./l P was reached. When phosphorus is increased further, sucrose concentration drops rapidly. The other nitrogen levels all show sucrose accumulation under conditions of phosphorus deficiency (0 me./l PO_4). This was followed by a rapid decrease in sucrose concentration until the 2.4 me./l phosphorus level was reached. This decrease was most marked in the 1.6 me./l nitrogen series. At this level of nitrogen, sucrose then accumulated as phosphorus concentration was increased beyond 2.4 me./l. In the two highest nitrate series, sucrose continued to decrease with increasing phosphate.

The sucrose response curves for D. glomerata showed sucrose accumulation at 0 me./l phosphorus only at the intermediate nitrogen concentrations (1.6 me./l NO_3 and 8 me./l NO_3). In both of these series, increasing phosphorus is accompanied by decreasing

sucrose concentration up to 2.4 me./l phosphorus. Further increases in phosphorus resulted in sugar accumulation. In both the 0 me./l nitrate and 32 me./l nitrate series, increase in phosphorus caused an initial increase in sucrose concentration. This was followed by a decrease in sucrose with increasing phosphorus to the 2.4 me./l P level (this was a marked decrease at the higher nitrogen level). When phosphorus concentration was increased further, sucrose again accumulated.

When the response curves of the last species, L. perenne, are examined (Figure 3.15 d), it is seen that for the three lowest nitrogen series, sucrose acccumualted when phosphorus was deficient. Furthermore, the accumulation is greater with increasing nitrogen levels. When 32 me./l NO_3 was present in the treatments, addition of phosphorus caused initial increase in sucrose concentration. Further additions of phosphorus were accompanied by a decrease in sugar concentration. Sucrose concentration in the two lowest nitrogen series increased when phosphorus was increased from 0.48 me./l to 2.4 me./l. Additional phosphorus resulted in decreasing amounts of sucrose.

Analysis of variance of phosphorus treatment versus sped versus species showed that in the 0 me./l

NO_3 level, phosphorus treatments did not account for a significant portion of the variability seen. The species were seen to be significantly different, and a significant treatmentxspecies interaction was present (both $p < 0.05$). In the remaining nitrogen levels, the effect of phosphorus treatments accounted for a significant proportion of the variability ($p < 0.01$, $p < 0.001$ and $p < 0.001$ respectively). When 1.6 me./l NO_3 was present in the treatments, there was no significant difference between the four species. There was, however, a significant ($p < 0.01$) species/treatment interaction. There was no significant difference between the species when 8 me./l NO_3 was present, and the species/treatment interaction effect was not statistically significant. At the highest nitrogen level, however, both species and species/treatment interactions accounted for significant proportions of the variability.

Effect of Nitrate at Each Phosphate Level

Summary and discussion of results

The results of this experiment which examined the response of three soluble sugars to different concentrations of both phosphorus and nitrate-nitrogen are summarised here as briefly as possible. The sugar responses to nitrate at each separate phosphorus level

will be discussed first.

(1) In the 0 me./l PO_4 level, the patterns of fructose and glucose responses of each species to increasing nitrate, however, tended to be much higher at each nitrate level than the glucose concentration. Further, the differences in fructose concentration were greater than the differences in glucose concentration.

(a) The reducing sugars of L. perenne and D. glomerata showed a similar pattern of initial sugar accumulation as the 1.6 me./l NO_3 level was approached. This was followed by a decrease in sugar concentration with increasing nitrate. Concentrations of nitrate above 8 me./l at this phosphorus level were accompanied by sugar accumulation.

(b) F. rubra and A. tenuis showed similar responses to increasing nitrate. They both showed an initial decrease with increasing nitrate to between 1.6 and 8 me./l NO_3. Above these levels there resulted an accumulation of sugar.

(2) In the 0 me./l PO_4 level, the patterns of sucrose response to nitrate of the four species were very similar. All four species showed initial and rapid accumulation of sucrose in nitrate levels up to 8 me./l in the cases of L. perenne, A. tenuis and D.

glomerata. F. rubra accumulated sucrose in nitrate levels up to 1.6 me./l. In this species, this accumulation was followed by a decrease in concentration to the 8 me./l NO_3 level. Further increase in nitrate resulted in sucrose accumulation. In the remaining species, increase in nitrate beyond 8 me./l resulted in a decrease in the concentration of sucrose.

(3) In the 0.48 me./l PO_4 levels, again the patterns of fructose and glucose responses of each species to increasing nitrate were similar. As in the previous phosphorus level, the fructose concentration at each nitrate level was greater than the corresponding glucose values, and the differences in fructose concentration between the species were greater than the differences in glucose concentration.

(a) L. perenne and D. glomerata showed similar reducing sugar concentration with increasing nitrate up to nitrate levels of 8 me./l. Above this nitrate concentration, sugar tended to decrease in amount or, in the case of the glucose response of D. glomerata, decrease in rate of accumulation.

(b) The response curves of the reducing sugars in F. rubra and A. tenuis showed initial decrease up to the 8 me./l NO_3 level (though the glucose response curve of F. rubra showed an increase in

concentration from 0 to 1.6 me./l NO_3 before a decrease as the 8 me./l NO_3 level was approached). Further increase in nitrate resulted in decreases in sugar concentration.

(4) The sucrose response curve at the 0.48 me./l PO_4 level was quite different from that at the 0 me./l PO_4 level. The response of the four species was roughly the same, the major differences being ones of concentration. The species showed initial decrease with increasing nitrate to the 1.6 me./l NO_3 level. Increases in nitrate beyond this concentration resulted in sugar accumulation. In D. glomerata, this accumulation was marked and continued to the highest nitrate level. In the remaining species, there was only a slight accumulation between 8 and 32 me./l NO_3.

(5) As in the other two phosphorus levels, the fructose and glucose response curves were very similar in the separate species. Additionally, the fructose concentrations of each species tended to be greater than the corresponding glucose concentrations, and the concentration differences between the species are greater with fructose than with glucose.

(a) The reducing sugar response curves for L. perenne and D. glomerata were similar in pattern.

That is, there was an initial increase in sugar concentration followed by a decrease in sugar between 1.6 and 8 me./l NO_3. Further increases in nitrate resulted in accumulation of these soluble sugars.

(b) F. rubra and A. tenuis showed a general increase in sugar concentration up to 8 me./l NO_3. The fructose response curve then showed a decrease in concentration with further increase in nitrate level. The glucose response curve showed a continued increase in sugar concentration above 8 me./l NO_3, though the rate of increase was lower.

(6) Sucrose concentration at the 2.4 me./l PO_4 level showed an initial decrease in F. rubra, A. tenuis and D. glomerata. This was followed by an increase in sucrose concentration which continued to 32 me./l NO_3 in F. rubra and D. glomerata, and which was followed by a marked decrease in concentration between 8 and 32 me./l NO_3 in A. tenuis. L. perenne was quite different in its sucrose response in that an initial sucrose increase up to the 1.6 me./l NO_3 level was followed by a continuous decrease to the highest nitrate level.

(7) In the highest phosphorus level (12.12 me./l), the reducing sugar responses of D. glomerata and L. perenne were similar. They both showed a decrease in

sugar concentration from 1.6 me./l NO_3 to 32 me./l NO_3. D. glomerata, however, showed an initial increase up to 1.6 me./l NO_3 while L. perenne showed a decrease over this same range. F. rubra showed an initial increase in sugar concentration up to the 1.6 me./l NO_3 concentration. This was followed by decreasing fructose concentration and increasing glucose concentration with increasing amounts of NO_3. (8) When 12.12 me./l PO_3 was present in each treatment, F. rubra and D. glomerata showed the same pattern of initial increase in sucrose concentration followed by a decrease to the 8 me./l nitrate level. A. tenuis and L. perenne both showed a decrease in sucrose concentration to this same level. Further increase in nitrate resulted in an accumulation of sucrose in all species.

In the above summary of results, pattern similarities were stressed. One of the major differences between species was that of the relative concentration of each soluble sugar. This will be taken into account in the discussion.

Effect of Phosphate at Each Nitrate Level

Summary: Soluble sugar response under conditions of phosphate deficiency

The responses of the soluble sugars in each species to phosphate content was presented in the 3-dimensional diagrams. The only disaccharide presented in detail in this study was the non-reducing sugar, sucrose. This sugar was seen to accumulate in A. tenuis as the phosphate content of the nutrient solution was reduced to 0 me./l (Figure 3.15a). This was true at all 4 nitrate levels. In F. rubra, this accumulation of sucrose was present at the 1.6, 8 and 32 me./l NO_3 levels only. When no nitrate was fed to this species, the sucrose concentration of the shoots decreased dramatically as added phosphate was reduced from 2.4 me./l to 0 me./l (Figure 3.15b). When the sucrose response curves of D. glomerata are examined (Figure 3.15 c), it is seen that the response of sucrose to decreasing phosphate depended upon the concentration of nitrate also present in the nutrient solution. When no nitrate was present, sucrose accumulated as phosphate decreased from 2.4 to 0.48 me./l, but the sucrose concentration then decreased as the level of phosphate in the nutrient solution decreased to 0 me./l. This pattern was also seen in the series of treatments containing 32 me./l NO_3 (the highest level). The

response curves of the 1.6 and 8 me./l NO_3 series were very similar. In these, sucrose accumulated dramatically as the phosphate concentration of the nurtient solution decreased from 2.4 to 0 me./l. The sucrose concentration of L. perenne (Figure 3.15 d) showed even more variability with changing nitrate level. When 0 and 1.6 me./l nitrate was present in the nutrient solution, sucrose accumulated only when phosphate was decreased from 0.48 to 0 me./l. In the series of treatments containing 8 me./l NO_3, sucrose continued to decrease as phosphate was reduced from 12.12 to 0 me./l. When each treatment contained 32 me./l NO_3, sucrose concentration decreased as phosphate concentration was reduced from 0.48 to 0 me./l.

Both of the reducing sugars of A. tenuis showed the same patterns at low phosphate levels, though the concentrations of the sugars were different (Figures 3.11 a and 3.13 a). In the series of treatments which contained no nitrate, reducing sugar concentration decreased as phosphorus concentration was reduced to 0 me./l from 0.48 me./l. At all other concentrations of nitrate, glucose and fructose accumulated in the shoots of this species as the phosphorus concentration was reduced from 0.48 to 0 me./l. When the response curves of the reducing sugars of F. rubra are examined, this accumulation of sugars in the 0 me./l PO_4 treatments

(ie. deficiency condition) was evident only in the response of fructose when 8 and 32 me./l nitrate was present in the nutrient solution. In the remaining treatments, fructose decreased in concentration from 0.48 to 0 me./l PO_4. Glucose decreased in concentration from 0.48 to 0 me./l PO_4 in all treatments. In the ones containing 8 and 32 me./l NO_3, glucose decreased in concentration from 2.4 to 0 me./l PO_4. This decrease was not so marked from 0.48 to 0 me./l.

The response curves of fructose and glucose were similar in pattern at each nitrate level in D. glomerata (Figures 3.11 c and 3.13 c). Again, the reducing sugars were different in actual concentration, fructose generally occurring in greater amounts than glucose. With the exception of the series of treatments containing 0 me./l NO_3, the reducing sugars decreased in concentration as phosphate was reduced from 0.48 to 0 me./l in the nutrient solutions. In the absence of nitrate, fructose accumulated in the shoots of this species as phosphorus was reduced from 2.4 to 0.48 me./l PO_4. Glucose accumulated with decreasing phosphorus from 2.4 to 0.48 me./l PO_4. The glucose concentrations in the 0.48 and 0 me./l treatments were the same. In addition, the degree of fluctuation in reducing sugar concentration with changing phosphate level was much less in the highest nitrate series. This

was also true of the reducing sugar response in L. perenne. In this species, in the absence of nitrate, both reducing sugars were seen to decrease in concentration as phosphorus was decreased from 12.12 to 0 me./l in the nutrient solutions. When 1.6 me./l NO_3 was present in each treatment, both glucose and fructose accumulated as phosphate was reduced from 0.48 to 0 me./l in those treatments containing 32 me./l NO_3, but fructose showed a tendency for accumulation over this reduction in phosphorus.

Discussion

As stated before, the factors which, on the basis of the literature on this subject, tend to favour a high content of soluble carbohydrates appear to be low amounts of growth in conditions in which photosynthesis can be maintained, very high rates of fertilisation which may cause a depression of growth due to high levels of NH_4 or NO_3 ions, nitrogen and phosphorus deficiencies, and low rates of amino acid/protein synthesis. Low soluble carbohydrate concentrations have been related to increased nitrogen fertilisation, decreased photosynthesis, increased rates of amino acid/protein synthesis, rapid assimilation of NH_4-N, and growth rates rapid enough to use the immediate products of photosynthesis as they are produced.

Considering the effect of phosphorus alone, Hewitt

and Smith (1974) noted that phosphorus deficiency usually causes the accumulation of reducing sugars and sucrose, among other changes in composition. Phosphorus deficiency has been found to cause an increase in sugar concentration in Mentha arvensis (Singh and Singh, from Lawanson, et. al. 1975). Phosphorus deficiency in oats is associated with an increase in disaccharide content (Hecht-Buchholz, 1969).

It appeared, then, that a large number of the sugar response curves in this experiment contradicted the findings that phosphorus deficiency generally causes the accumulation of reducing sugars and sucrose. It is apparent, therefore, that the effect of phosphorus deficiency on the soluble sugar content depends to some extent on the nature of the plant material used as well as the particular experimental conditions. The accumulation of soluble sugars under conditions of nutrient deficiency, when it occurred, was most likely due to a depression of growth. The results presented in Chapter 2 showed that the four experimental species each showed very low yields at the lowest concentration of nutrients, when yield was measured in terms of shoot dry weight. In fact, the greatest percentage reduction from maximum yield in all of the species resulted from growth in the lowest concentration of modified Hoagland's solution. Another

contributing factor may be a low rate of amino acid/protein synthesis in these cases. This aspect will be discussed in the next chapter. The factors contributing to the decrease in soluble sugar concentration under conditions of apparent phosphorus deficiency cannot be explained in terms of the growth response to the different nutrient concentrations. In terms of the factors affecting soluble sugar concentration which have been discussed so far, increased rates of amino acid/protein synthesis, increased nitrogen fertilisation and decreased photosynthesis may have played a part in the observed response. As stated before, a detailed examination of the amino acid content of the four species in this experiment will be presented in the next chapter. Examining the cases in which this decrease in soluble sugar concentration occurred, it is found that sucrose concentration decreased under conditions of very low phosphorus content in F. rubra only in the absence of nitrate. In D. glomerata, the content of sucrose in the shoots decreased under these same phosphorus levels when nitrate was absent and when nitrate was present in its highest concentration in this experiment (32 me./l). In L. perenne, only when nitrate was present in the amounts of 8 and 32 me./l, did sucrose content decrease with decreasing phosphorus from 0.48 to 0

me./l PO_4.

When the reducing sugars are examined, this decrease in concentration is seen in A. tenuis in the absence of nitrate. In F. rubra, glucose concentration decreased in these low phosphorus treatments in all nitrate levels, and fructose content decreased only in the low nitrate treatments (0 and 1.6 me./l NO_3). This reducing sugar decrease was present in D. glomerata at all nitrate levels except that in which nitrate was absent. Finally, in L. perenne, reducing sugars showed a decrease in concentration in the lowest phosphorus levels in the absence of nitrate and when nitrate was present in the amount of 8 me./l in each treatment. In the highest nitrate level, only glucose showed this decrease, and the accumulation of fructose in the absence of phosphorus was only slight.

Under conditions where nitrogen and phosphorus were not present in the nutrient solution, decreased photosynthesis would probably account for the low concentration of the soluble sugars studied; increased photosynthesis and amino acid/protein synthesis are very unlikely. Nitrogen deficiency is known to dominate the effects of the other elements, and can have overriding control of growth. Additionally, the amounts of proteins and protein-rich tissues, ie. phloem, are reduced under conditions of nitrogen deficiency as is

chlorophyll production (Hewitt and Smith, 1974). It is likely, however, that carbohydrates will accumulate until the deficiency is severe enough to affect the chloroplasts. The decrease in soluble sugar when both of these elements are absent is not, therefore, unexpected. This occurred in sucrose and the reducing sugars of F. rubra, in sucrose in D. glomerata, and in the reducing sugars of A. tenuis and L. perenne.

When nitrate was present in the amounts of 8 and/or 32 me./l (the two highest values), this decrease was seen in D. glomerata and L. perenne in sucrose and both reducing sugars. In F. rubra, this decrease in the presence of relatively high nitrate levels is seen only in the response of glucose. In these cases, the effect may be partly due to the effect of growth and/or amino acid/protein synthesis. This may be clarified in the next chapter. If this was the case, nitrogen would be the limiting element with respect to phosphorus. In other words, even at low phosphorus levels increasing amounts of nitrogen have an overriding effect on yield. If the soluble sugar decrease in the presence of high nitrate levels was due to a negative effect such as a decreasin photosynthesis, it would be apparent that phosphorus was the limiting element, ie. in the absence of phosphorus, increasing increments of nitrogen would have no direct effect on yield.

Summary: Soluble sugar response to high levels of phosphate

The next aspect of this experiment to examine is the reaction of the species to high levels of phosphate: in this examination, the response of the soluble sugars to increasing phosphorus from 0.48 to 12 me./l at each nitrate level. The response of the dissacharide, ie. sucrose, to this increase in A. tenuis depended somewhat on the nitrate level of the treatments. In the absence of nitrate, sucrose decreased over this range of phosphorus concentration. In the presence of 1.6 and 8 me./l NO_3-N, sucrose accumulated in the 2.4 me./l phosphate treatments and decreased in concentration in the 12 me./l phosphate treatments. In the treatments containing the highest concentrations of nitrate, sucrose decreased in concentration in the 2.4 me./l PO_4 treatment and accumulated when the treatment contained 12 me./l PO_4. E. rubra showed an increase in sucrose concentration in the 2.4 me./l PO_4 treatment when no nitrate was present in the nutrient solution. The amount of this sugar then decreased in the 12 me./l PO_4 treatment. When 1.6 me./l NO_3 was added to the treatments, sucrose decreased in the 2.4 me./l PO_4 treatment and then accumulated when the phosphate content of the nutrient solution was 12 me./l. In the remaining high nitrate treatments,

sucrose continued to decrease from 0.48 to 12 me./l PO_4.

D. glomerata showed a decrease in sucrose concentration in the 2.4 me./l PO_4 treatments and an accumulation of sucrose in the 12 me./l PO_4 treatments at all nitrate levels except that where nitrate was absent. In this series of treatments, increasing phosphorus resulted in decreasing sucrose concentration from 0.48 to 12 me./l PO_4. In L. perenne in the two lower nitrate series of treatments, ie. those containing 0 and 1.6 me./l NO_3, sucrose increased from 0.48 to 2.4 me./l PO_4. When no nitrate was present, sucrose then increased slightly with increasing phosphorus, while in the 1.6 me./l NO_3 treatments, sucrose showed a tendency to decrease. In the remaining treatments, sucrose continued to decrease as phosphorus increased from 0.48 me./l to 12 me./l. Although the sucrose concentrations differed considerably between the species, especially at the extremes of phosphorus concentration, the results showed that in general around the levels of 0.48 and 2.4 me./l PO_4, the sucrose contents of A. tenuis and F. rubra were lower than those of D. glomerata and L. perenne over this same range irrespective of nitrate level. Additionally, L. perenne showed lower levels of sucrose content in treatments containing greater than 0 me./l phosphate.

As stated before, the patterns of fructose and glucose in each series of treatments were very similar in A. tenuis. In the absence of nitrate, the reducing sugars showed a decrease in the 2.4 me./l PO_4 treatments and an accumulation in the 12 me./l treatments. In all other nitrate levels, fructose and glucose accumulated in the 2.4 me./l PO_4 treatments and decreased in concentration when 12 me./l PO_4 was present. With one exception, the patterns of reducing sugar response in F. rubra were the same as those of A. tenuis. In the series containing 32 me./l NO_3, sucrose showed a small increase in concentration in the 12 me./l PO_4 treatment over that seen in the 2.4 me./l PO_4 treatment.

D. glomerata showed the same pattern of fructose decrease at the 2.4 me./l PO_4 treatments and accumulation when phosphorus concentration was increased to 12 me./l in the 0, 8, and 32 me./l NO_3 series. This was true of glucose in the 0 me./l NO_3 treatments. When 1.6 me./l NO_3 was present in the nutrient solutions, both reducing sugars showed an accumulation in the 2.4 me./l PO_4 treatments, and a decrease in those containing 12 me./l PO_4. Glucose continued to decrease from 0.48 me./l PO_4 to 12 me./l PO_4 in the treatments containing 8 me./l NO_3. In those containing 32 me./l NO_3, glucose accumulated over this

range in the highest nitrate series. Unlike the case in D. glomerata, both reducing sugars showed the same patterns of response in L. perenne. In the absence of nitrate, the reducing sugars showed an increase over the 0.48 to 12 me./l PO_4 range. When 1.6 me./l NO_3 was present in the nutrient solutions, fructose and glucose accumulated in the 2.4 me./l PO_4 treatments. In the remaining treatments, reducing sugars decreased in concentration over the 0.48 to 12 me./l PO_4 range.

When the reducing sugar content is considered in addition to the pattern of response, F. rubra reached the highest fructose content of all of the species. This high content was reached when 2.4 me./l phosphate was present in each treatment. The highest fructose concentration at each nitrate level occurred in treatments containing this amount of phosphorus, and was greatest at 8 me./l NO_3. In A. tenuis, with the exception of the case in which nitrate was absent, the highest fructose content occurred again when 2.4 me./l PO_4 was present in the nutrient solutions, though the concentrations of fructose in this species were considerably lower than those of F. rubra. In L. perenne and D. glomerata, again with the exception of the treatments containing no nitrate, the highest fructose concentrations generally occurred at the much lower 0.48 me./l phosphate level.

The glucose content of all four species was seen to be much lower than that of fructose (Figures 3.13 a-c). As in the response of fructose to nitrate and phosphate, F. rubra reached the greatest glucose concentrations of the four species. Here, the highest glucose content occurred in treatments containing 2.4 me./l PO_4, though only in the 8 and 32 me./l nitrate treatments. The highest glucose content occurred when the nutrient solution contained 32 me./l NO_3. A. tenuis, with the exception of the series of treatments without nitrate, also showed the greatest glucose content in those treatments containing 2.4 me./l phosphate. Though the glucose responses of D. glomerata and L. perenne were more variable than those of fructose, and of the glucose responses of the other two species, they generally showed lower concentrations of glucose than A. tenuis and F. rubra especially in the higher nitrate levels. Additionally, the higher glucose contents in L. perenne were seen in the treatments containing 0.48 me./l PO_4 in the 8 and 32 me./l NO_3 treatments as in the fructose treatments. The phosphate levels which resulted in the highest glucose content in D. glomerata varied from 12 me./l to 2.4 me./l to 0.48 me./l to 12 me./l when nitrate was increased from 0 me./l to 32 me./l. However, the glucose concentrations of the 0.48 me./l PO_4 treatments were generally higher

than those of A. tenuis at the same phosphate level and higher than those of F. rubra when 8 me./l NO_3 was also present. The reducing sugars were different from sucrose in concentration in that A. tenuis and F. rubra (with the exception of the treatment without nitrate in F. rubra) showed highest sucrose concentration when no phosphate was present. This was true of L. perenne and D. glomerata when 1.6 and 8 me./l NO_3 was present in the nutrient solution. The treatments containing no nitrate were, again, exceptions, and in the treatments containing 32 me./l NO_3 (the highest level), sucrose was highest in the 0.48 phosphate treatments.

These differences between the reducing sugars and sucrose in the phosphate level which results in the highest sugar concentration may be due to the previous observation that high reducing sugar content is associated with active growth (Green and Beard, 1969). Therefore, high concentrations of glucose and fructose would not be expected in treatments without nitrate or phosphate irrespective of species differences. As seen in the predeeding summary, the responses of the soluble sugars to additional increments of phosphate become more complex than the straight forward case of phosphorus deficiency (ie. 0 me./l PO_4 in the nutrient solutions).

The discussions in this chapter have described the

effects of phosphate, ammonium and nitrate ions in different experiments on the soluble sugar content of the four experimental species. Where possible, these reponses were related to growth and nutrient deficiency effects or the effects of toxicity due to high ion concentrations. Another important factor related to the soluble sugar content of plants has been identified as the amino acid status of the plant. The next chapter examines this aspect of the response of the four species to different nutrient programs. The last chapter will, therefore, incorporate this additional information.

Thus far, it has been seen that these species differ in their growth response, in terms of shoot dry weight, to increasing concentrations of modified Hoagland's solution, and the content of soluble sugars in their shoots also differs with increasing concentrations of this same solution. On the basis of these results, the species of oligotrophic environments showed differences in pattern of response from the species typical of eutrophic environments. Experiments in which only one or two nutrient ions varied from treatment to treatment showed different results from those of the preliminary, complete nutrient solution experiment. However, it was seen that the soluble sugars in each experiment generally varied

significantly with treatment effects, and in many cases the variability due to species differences and species/treatment interactions (indicating different sorts of response between the species) was statistically significant. In addition, although the different experiments showed different results, in many cases the species of oligotrophic environments, A. tenuis and F. rubra, responded differently as a group from those of eutrophic environments, D. glomerata and L. perenne. This would suggest that the responses seen in the complete nutrient experiment could be due to the cumulative effect of a number of factors, and if any single ion has a large effect on the pattern of soluble sugar response, it would be mainly in the areas of a severe deficiency resulting in a limiting effect, or an extreme surplus causing toxicity.

CHAPTER 4

AMINO ACID CONTENT AND NUTRIENT LEVEL

There is an enormous variety of soluble nitrogen-containing compounds in plants. Amino acids, nucleic acids, amides, nitrate, proteins, nitrite, ammonia, purines, coenzymes (including NAD and NADP), hydroxymates, amines, nitrosamines and pyrimidines are but a few of them. It has been shown that these compounds can show considerable changes in concentration in certain plants when these plants are grown under conditions in which there is a deficiency or an excess of mineral nutrients. Indeed, the assimilation of ions of ammonium and nitrite into organic form in the root system is reflected in the organic nitrogen compounds in the plant's xylem sap (Street and Öpik, 1971). When the literature allows comparison of changes in amino acids and amides between species, the pattern of amino acid changes caused by variations in the concentration of a given element is quite different for different species (Hewitt and Smith, 1974). Therefore, as this study was concerned with the responses of certain soluble carbohydrates to nitrogen and phosphorus nutrition in four species differing in their growth responses to different nutrient treatments, and as rates of amino acid/protein

synthesis are intimately involved with growth, it seemed necessary to study the range of variation in response of amino acids in these species to full nutrient solution treatments and to the effects of NO_3-N, NH_4-N and PO_4.

The measurements of the amino acids soluble in 60% and 80% ethanol in the shoots of the four species studied was carried out in one of the experiments investigating the response of these species to different levels of modified Hoagland's solution. These amino acids were also measured in the experiments in which the kind and amount of nitrogen supplied to the treatments varied and in which the effect of phosphorus at different nitrate levels was investigated.

Analysis of Ethanol Soluble Amino Acids

The method used to measure the soluble amino acids of *L. perenne*, *F. rubra*, *D. glomerata* and *A. tenuis* was a modification of a qualitative method using the ninhydrin reaction (Plummer, 1971). Ninhydrin reacts with all α-amino acids between pH 4 and 8 to yield a compound which is purple in colour. This reaction is very sensitive, and the intensity of colour can be used to measure the amino acids quantitatively with a spectrophotometer. As not all amino acids give the same intensity of colour, this method is usually used for the quantitative determination of single amino acids, the colour intensity of the sample being compared to that of a range of known concentrations of the same amino acid acting as a standard. Because the large number of samples in each experiment made the analysis of separate amino acids a practical impossibility, this method was further modified, so that the total soluble amino acids in each sample were measured in terms of the response of glutamic acid standards to the ninhydrin reaction. The same method was used throughout this study. Although it could be argued that the measured amount of amino acids present in each sample would vary according to the particular standard used in the quantification, the relative amounts of amino acids per gram shoot dry weight of each species and their

responses to nutrient level will be correct.

The actual method used was the following. Two 2-ml aliquots of each of the same ethanolic extractions used for soluble sugar analysis were taken for amino acid analysis. One was a "blank" to account for the chlorophyll content, and the other was the sample to which the ninhydrin reagent was added. This reagent was freshly prepared for each analysis and consisted of 0.8 g ninhydrin and 0.12 g hydrindantin dissolved in 30 mls of methyl cellosolve (ethylene glycol mono-methyl ether) with an added 10 mls of acetate buffer (pH 5.5). Two mls of buffered ninhydrin reagent were added to one 2 ml sample, and 2 mls of the reagent minus ninhydrin and hydrindantin were added to the chlorophyll blank. All of the samples were heated in a boiling water bath for 15 minutes. These were then allowed to cool to room temperature. Three mls of 50% ethanol were added to each sample, and the extinction was read in a spectrophotometer at 570 nm after 10 minutes. Appropriate concentrations of glutamic acid standards were analysed with the samples each time an analysis was run. A regression line was calculated for each series of standards: concentration of glutamic acid against extinction at 570 nm. The amount of amino acids in each sample was then determined from the sample extinction using this line. The regression lines for

glutamic acid were calculated in the same way as those of the standard sugars in Chapter 3. The results of each experiment will be discussed separately followed by a comparison of the results of the amino acid analyses of all of the experiments.

Effects of Different Concentrations of Complete Nutrient Solution on Ethanol Soluble Amino Acids

The first experiment in which soluble amino acids were measured was described in detail in Chapter 2. In this experiment, seeds of the four species under investigation were sown on non-absorbent cotton wool in identical plastic pots. There were 4 replicates of each species in each of 9 levels of a modified Hoagland's solution. These levels corresponded to 0.1%, 10%, 31%, 68%, 100%, 150%, 200%, 250% and 300% concentrations of the basic solution (page 12). This experiment was done in a glasshouse during March and April 1975. Growth in the treatments took place over 5 weeks. The solutions were renewed twice weekly. The methods of harvesting, pre-treatment and extraction were described in detail in Chapter 3. The method of amino acid analysis was described in this chapter.

After analysis, the results were standardised and presented in the form of mg amino acid per gram shoot dry weight. These data are presented in graphical form in Figure 4.1. The results are not discussed

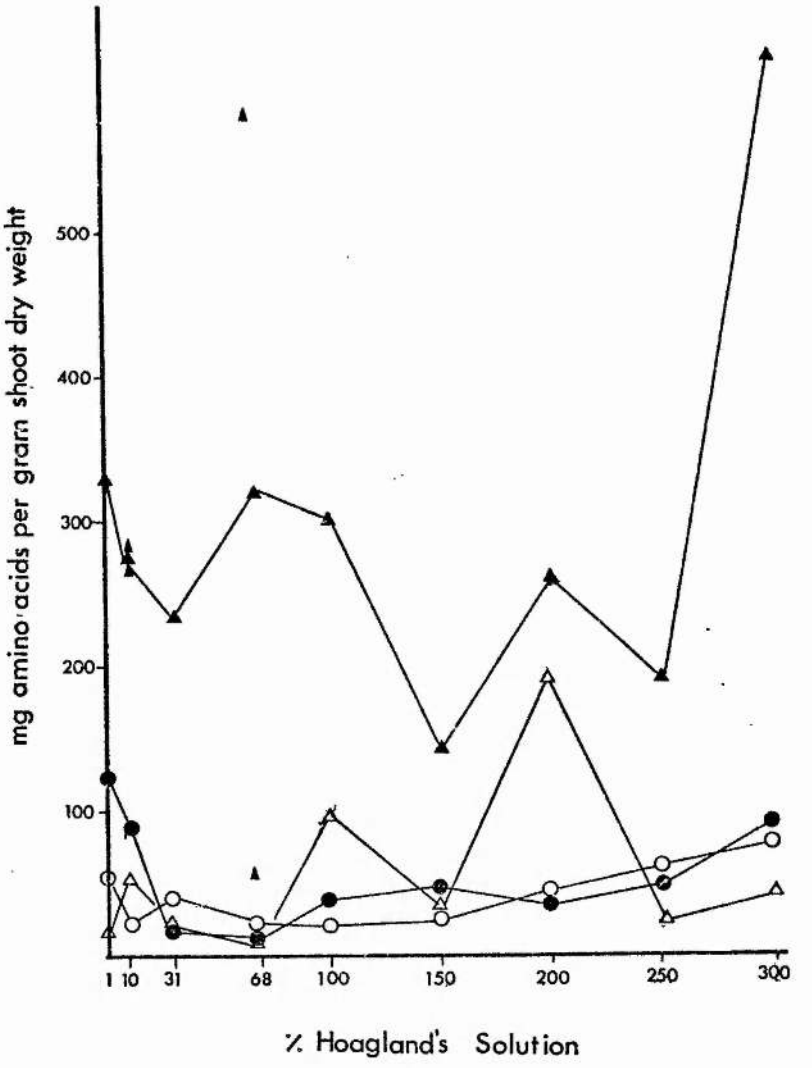


Figure 4.1: Amino acid concentration of 4 species grown for 5 weeks in treatments of 9 different concentrations of a modified Hoagland's solution. Points connected by lines are the average of 4 replicates. Maximum and minimum values are shown (amino acids measured using a glutamic acid standard).

○ = L. perenne ● = D. glomerata
▲ = A. tenuis △ = F. rubra

statistically, because a number of values of individual replicates will not be included in the analysis. This is due to two factors: either the replicates in question were lost due to spillage or some of the sample was lost because "bumping" occurred in the test tubes during heating in the boiling water bath. This would, of course, make quantitative measurements impossible. These problems were overcome in later analyses. The results are presented here to give some idea of the trends of the responses of amino acids as a whole to different levels of complete nutrient solution. The limitations of these data will be borne in mind when the results are discussed. Because standard deviations would be meaningful for some of the points in Figure 4.1 and not for others, the maximum and minimum points of the replicates will be shown, and the means of the existing replicates will be connected by lines in the usual way.

The effect of increasing concentrations of complete nutrient solution of total amino acid concentration in the 4 species can be summarised quite quickly. The responses of L. perenne and D. glomerata were very similar. The amino acids in thses species showed an initial decrease in concentration with increasing nutrient strength to between the 31% and the 68% levels of the modified Hoagland's solution. This

was followed by a gradual accumulation of amino acids as nutrient concentration increased. The concentrations of all of the amino acids of these two species range between 0 and 125 mg amino acid per gram shoot dry weight. This range was much lower than that of A. tenuis. In that species, the amino acid concentration ranged between 140 and 627 mg amino acid per gram shoot dry weight. A. tenuis also showed a decrease in amino acid concentration from the 0.1% to the 31% nutrient levels. This was followed by an accumulation of amino acids as the 68% level of nutrients was approached. As nutrient strength was increased above this level, the amino acids in this species showed marked fluctuations in concentration, ending in a final dramatic accumulation of amino acids as the 300% nutrient level was approached. F. rubra showed the same pattern of fluctuations in amino acid concentration with increasing nutrient level as A. tenuis, but the actual concentrations reached were on the whole lower. The amino acid concentrations of F. rubra ranged from 14 to 199 mg amino acid per gram shoot dry weight.

At first sight, it seemed that the marked fluctuations seen in the response of amino acid concentration to increasing nutrient level in A. tenuis and F. rubra were due to the fact that more of the treatments of these two species had missing replicates

than those of the other species. However, since different replicates were missing in each of these two species, it would be likely that the patterns shown in Figure 4.1, or ones very much like them, are in fact the actual response curves of amino acids in these species. Furthermore, since both the growth patterns of these species and the responses of their soluble carbohydrate concentrations to increasing nutrient level were very similar, it would not be unreasonable to expect their amino acid response curves to be similar as well.

Although it cannot be statistically tested, it seems that once again the responses of D. glomerata and L. perenne are different from those of F. rubra and A. tenuis. D. glomerata and L. perenne, the species which showed much greater yield, showed consistently low concentrations of amino acids and a tendency to accumulate amino acids only when very high or very low concentrations of the modified Hoagland's solution were used. A. tenuis and F. rubra showed very similar amino acid response curves. These were the species which were seen to have lower yields than either of the other two species.

Effects of Solutions of Different Levels of Nitrogen on Amino Acid Concentration

This experiment was described in detail in Chapter 3. It was designed to test the responses of the 4 experimental species to two different forms and several different concentrations of nitrogen. The experiment was divided into 3 sections. In one, nitrate was the only form of nitrogen in the treatments. There were 6 treatments, 2 replicates each, and the levels of nitrate in me./l were 4, 8, 16, 24, 32 and 48. In the second section, there were 6 treatments in which ammonium was the only nitrogen source; the concentrations of the NH_4 ions were in the same me./l concentrations as the NO_3 ions in the previously described treatments. Again, there were 2 replicates. The third phase of the experiment used a single level of nitrogen-containing ions (ie. both NO_3 and NH_4 ions), but the proportions of the two ions were different in each treatment. The me./l concentrations of each ion, NO_3 and NH_4, were 24 and 0, 20 and 4, 16 and 8, 12 and 12, 8 and 16, 4 and 20, and 0 and 24. There were 2 replicates of each species in each treatment. All the other macronutrients and micronutrients were at a constant level in each treatment. The exact concentrations of the other

nutrients are given in Chapter 3. The four species were grown in the different nutrient solutions for 6 weeks during July and August 1975. At harvest only the shoots were taken. The harvesting procedure and the pre-treatment of samples were described in detail in Chapter 3. The analysis of amino acids was described at the beginning of this chapter.

Once again, the results are presented in graphical form in Figures 4.2 a-c. Figure 4.2 a shows the amino acid concentration of the 4 experimental species in the 6 treatments of different NO_3-N concentration. L. perenne and D. glomerata showed much the same pattern. Both of these species showed an initial accumulation of amino acids as the amount of NO_3-N was increased above 4 me./l. There were some minor fluctuations in both species as nitrate was increased to 24 me./l, but the general levels of amino acid concentration in both species was maintained at a level just below 25 mg per gram shoot dry weight. L. perenne then showed an accumulation of amino acids as 32 me./l NO_3-N was approached. This brought the amino acid concentration in this species to just above 25 mg per gram shoot dry weight. This concentration was maintained at the highest nitrogen level. D. glomerata showed a decrease in amino acid concentration between the 24 and 32 me./l NO_3-N levels to a concentration of about 12 mg amino

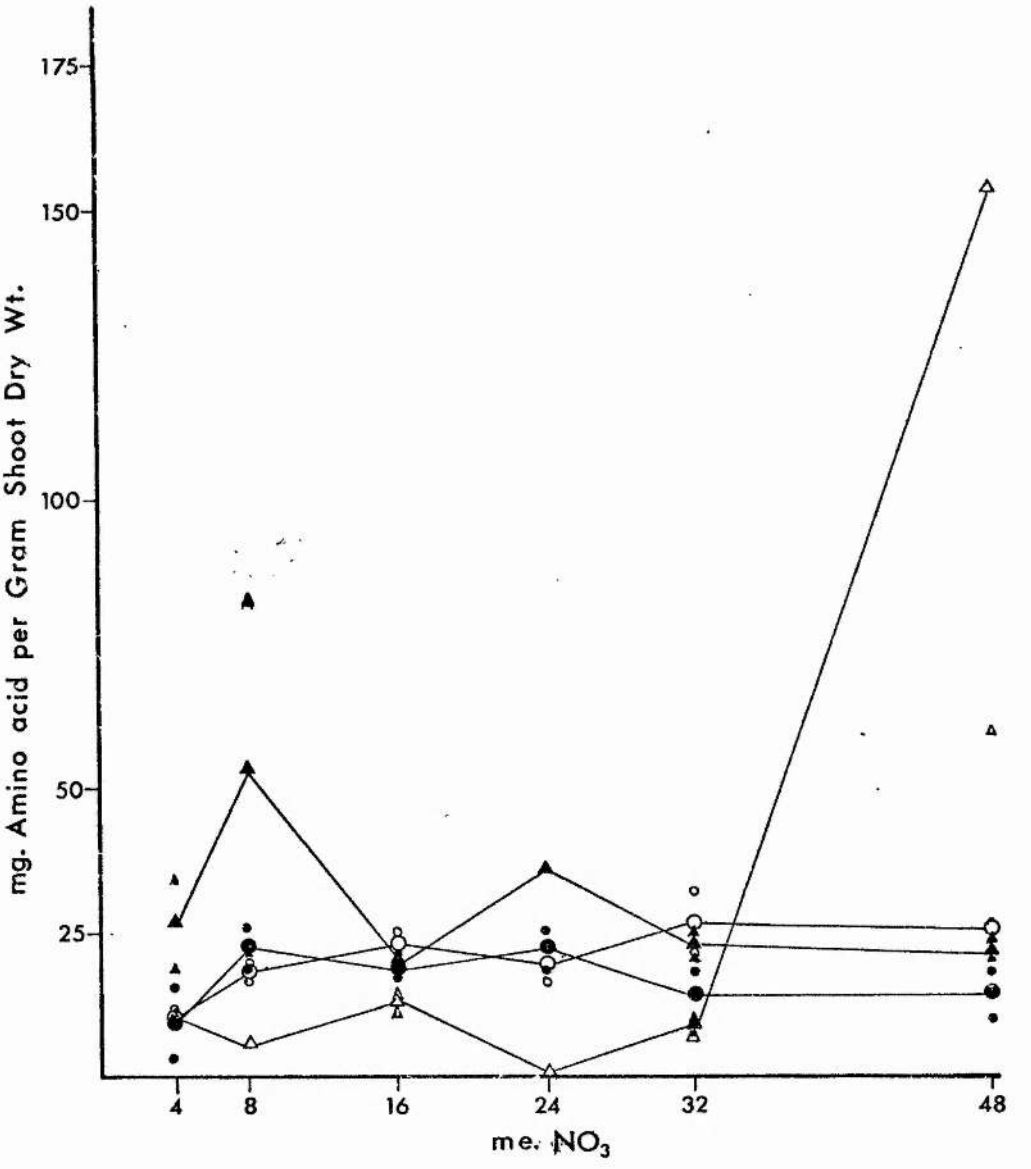


Figure 4.2a: Amino acid concentration of 4 species grown for 6 weeks in treatments of 6 different concentrations of NO_3-N. Amino acid content is measured in terms of mg amino acid per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

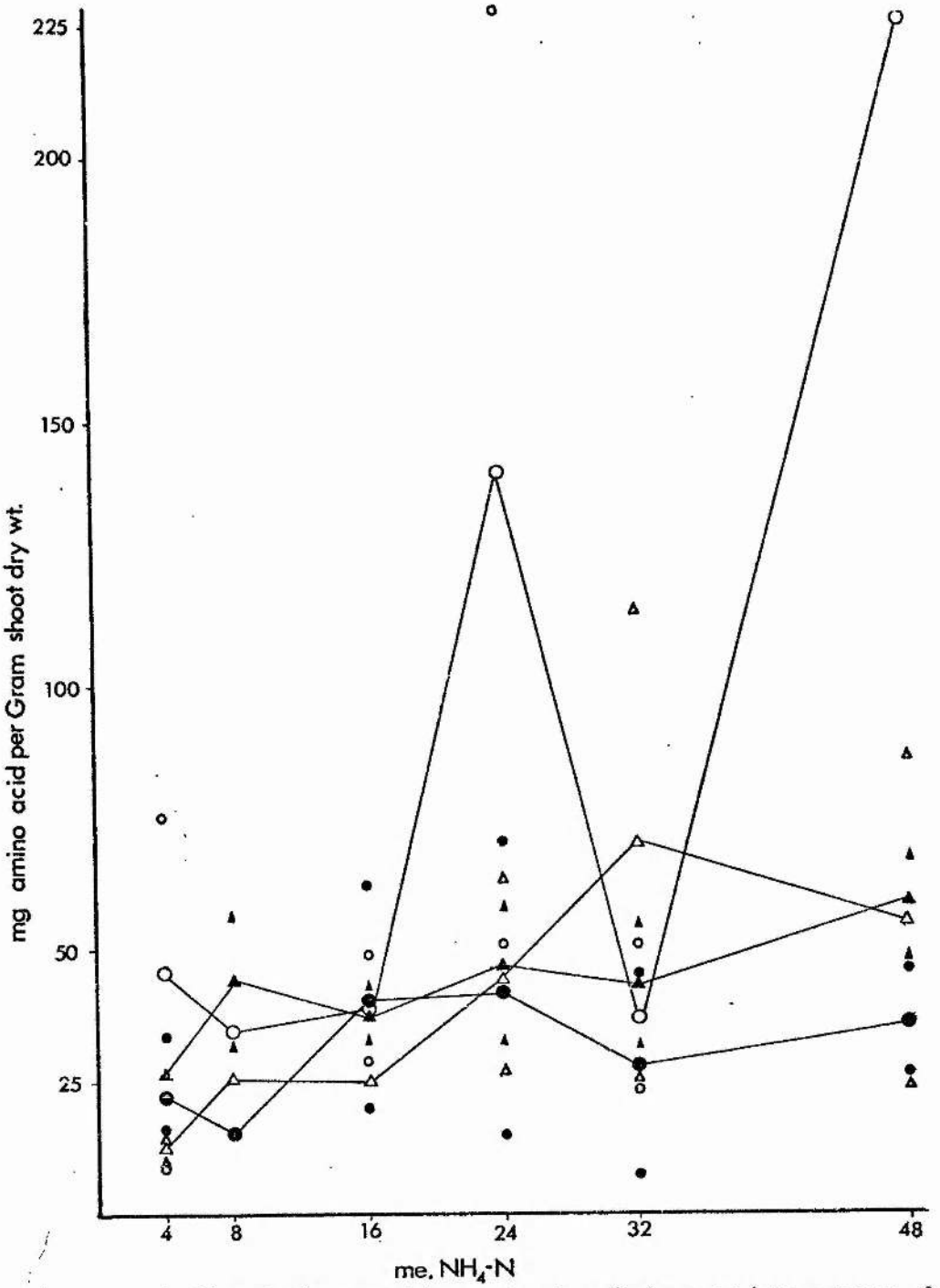


Figure 4.2b: Amino acid content of 4 species grown for 6 weeks in treatments of 6 different concentrations of NH_4-N. The amino acid content is measured in terms of mg amino acid per gram shoot dry weight using a glutamic acid standard. The means of the two replicates are connected by lines, and the actual values of the replicates are shown (the maximum value of *L. perenne* at 48 me./l NH_4-N is outside the range of this graph).

○=*L. perenne* ●=*D. glomerata*
▲=*A. tenuis* △=*F. rubra*

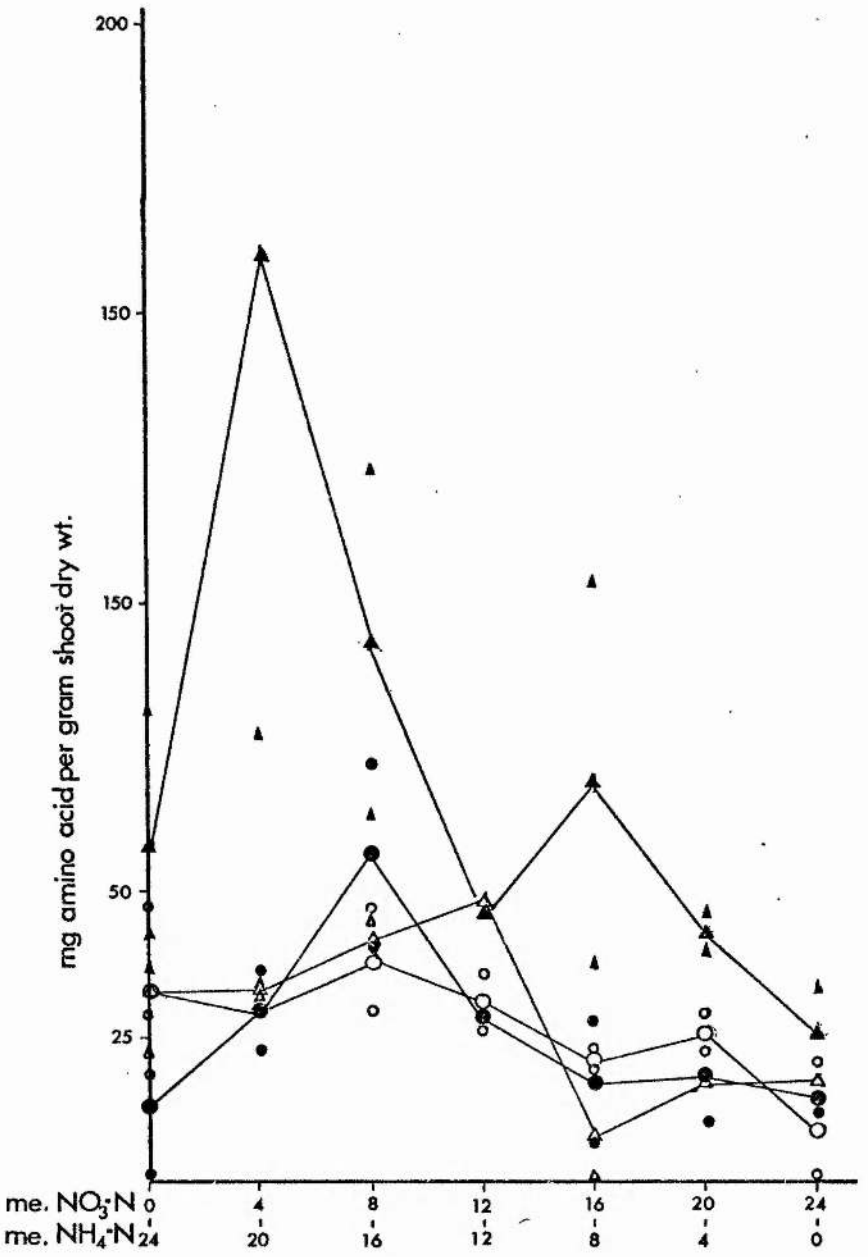


Figure 4.2c: Amino acid content of 4 species grown for 6 weeks in 7 treatments of a complete nutrient solution with a nitrogen concentration of 24 me./l in which the proportions of NO_3-N and NH_4-N varied from treatment to treatment. Amino acid content was measured in terms of mg amino acid per gram shoot dry weight using a glutamic acid standard. The means of the two replicates are connected by lines, and the actual values of the replicates are shown (the maximum value of A. tenuis at 4 me./l NO_3-N is outside the range of the graph).

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

acid per gram shoot dry weight. As in L. perenne, this concentration was maintained at the highest nitrogen level.

In general, A. tenuis showed higher amino acid concentration than L. perenne or D. glomerata except at the 32 me./l NO_3-N level. In that treatment, amino acid concentration fell to about 25 mg per gram shoot dry weight. This concentration was maintained in the treatment with the highest nitrate level. Below 32 me./l NO_3-N, A. tenuis showed a pattern of marked fluctuations with increasing NO_3-N level, beginning with an initial increase in amino acid concentration with increasing NO_3-N. A. tenuis showed a greater difference between replicate values at the two lowest nitrate levels. These differences were greater than those of L. perenne or D. glomerata at any nitrate level.

F. rubra also showed more marked fluctuations of amino acid concentration than either L. perenne or D. glomerata In addition, this species was quite different from A. tenuis in the pattern of amino acid response to increasing NO_3-N. The fluctuations in amino acid concentration in F. rubra were completely out of phase with those of A. tenuis. This species generally showed lower levels of amino acids than the other species. There was also an initial decrease in amino

acid concentration as NO_3-N was increased above the lowest level. In the treatment containing 48 me./l NO_3-N, F. rubra showed an exceptional accumulation of amino acids. Although the lowest value of the two replicates indicated an amino acid accumulation, the great difference in values of the two replicates casts doubt on the accuracy of the actual mean value as an indicator of the amino acid response of this species to that particular nitrate level.

The responses of the amino acid concentrations of the 4 experimental species to increasing levels of ammonium-nitrogen are illustrated in Figure 4.2 b. It is seen from this graph that A. tenuis and F. rubra both showed a general trend for amino acid accumulation with increasing NH_4-N. In the treatments containing concentrations of NH_4-N below 32 me./l, F. rubra again showed lower amounts of amino acids than A. tenuis. The 32 me./l NH_4-N treatment showed an accumulation of amino acids followed by a decrease in amino acid concentration in the 48 me./l NH_4-N treatment. However, the replicates of F. rubra grown in each of the highest ammonium-nitrogen treatments contained very different amounts of amino acids, and therefore, the values of the means of these replicates may not give an accurate picture of the amino acid response in this species.

D. glomerata and L. perenne both showed an initial

decrease in amino acid concentration from the 4 me./l to the 8 me./l NH_4-N level. This was followed by an accumulation of amino acids with increasing NH_4-N up to 24 me./l NH_4-N. This was a dramatic accumulation in L. perenne, the mean of the replicates reaching 140 mg amino acid per gram shoot dry weight. However, as was the case with F. rubra, in one of the NO_3-N treatments, although the lowest value of the two replicates still indicated an increase in amino acid concentration, the variability between the two replicates made the actual mean value unreliable. The same was true of the marked accumulation of amino acid in L. perenne between the 32 and 48 me./l NH_4-N levels. The accumulation of amino acids in D. glomerata at the 24 me./l NH_4-N level reached only 44 mg per gram shoot dry weight. Amino acids then decreased in concentration in this species as NH_4-N was increased as far as the 32 me./l NH_4-N level. Thereafter, amino acids accumulated with increasing NH_4-N as the 48 me./l level was approached.

The last of the figures for this experiment, Figure 4.2 c, shows the response of amino acids to 24 me./l nitrogen with different proportions of NO_3-N and NH_4-N. Examining the data species by species, A. tenuis showed the highest concentrations of amino acids of the 4 species over the entire range of treatments. Although it showed a comparatively low concentration of amino

acids in the treatment containing 24 me./l NH_4-N and no NO_3-N, there was a definite trend in this species for amino acid accumulation in those treatments with a higher proportion of ammonium-nitrogen than nitrate-nitrogen. This pattern is still apparent in spite of the great variability between replicates.

In this series of treatments, F. rubra showed much smaller differences between the replicates than in the treatments using only ammonium-nitrogen or nitrate-nitrogen. The greatest difference between the replicates occurred in the treatments with 24 me./l NH_4-N and no NO_3-N. The maximum concentration of amino acids in this species occurred in response to the treatment containing 12 me./l of both NO_3-N and NH_4-N. With increasing proportion of NH_4-N, amino acid concentration decreased gradually to a level of about 32 mg per gram shoot dry weight. With increasing proportion of nitrate-nitrogen, there was a more pronounced decrease in amino acid concentration. This decrease brought the amino acid concentration to just under 18 mg amino acid per gram shoot dry weight.

The patterns of amino acid response in D. glomerata and L. perenne were very similar and can be discussed together. In both these species, the greatest concentration of amino acids occurred in the treatment with 8 me./l NO_3-N and 16 me./l NH_4-N. With an

increasing proportion of NH_4-N in the treatments, the amino acid concentration of D. glomerata decreased continuously to a value of 14.6 mg per gram shoot dry weight in the treatment containing 0 me./l NO_3-N and 24 me./l NH_4-N. In L. perenne, this decrease in amino acid concentration was more gradual and only decreased to a level of 32.7 mg per gram shoot dry weight. With increasing proportion of NO_3-N, both of these species showed a gradual decrease in amino acid concentration to the 24 me./l NO_3-N treatment. The level reached by D. glomerata was 16 mg amino acid per gram shoot dry weight.

When the data from the three parts of this experiment were combined and analysed with the GLIM analysis of variance described in Chapter 3, it was found that 40% of the variability could be explained. This analysis is presented in Table 4.1.

Table 4.1: GLIM analysis of variance of the effect of kind and quantity of nitrogen supplied to 4 grass species on the concentration of total α-amino acids (mg amino acids per gram shoot dry weight). The values used were the means of two replicates.

Source of Variance	Sum of Squares	d.f.	M.S.	F	
Species	1.45	3	0.48	2.9	*
NO_3-N	0.63	1	0.63	3.9	*
NH_4-N	4.56	1	4.56	27.9	***
Replicates	0.04	1	0.04	4.09	N.S.
Species x Replicates	0.64	3	0.21	1.3	N.S.
Species NO_3-N	2.02	3	0.67	4.1	**
Species NH_4-N	2.12	3	0.71	4.3	**
Residual	22.25	136	0.16		
TOTAL	33.71	151			

33.99% of the variability is explained
* P<0.05, ** P<0.01, *** P<0.001
N.S. - not significant

This reveals some interesting clues about the nature of the responses of amino acid concentrations to the kind and quantity of nitrogen given to 4 different species. Firstly, there is evidence of a significant difference between the responses of amino acids of the 4 species. Secondly, there is a significant effect caused by the different nitrate-nitrogen treatments and a highly significant effect caused by the ammonium-nitrogen treatments. It is interesting to note that despite the great differences between some of the replicate pairs, the analysis of variance showed that the replicates fail to account for a significant portion of the variability in the experiment.

Additionally, there is no significant effect due to interaction between the species and replicates. The interactions of species and ammonium-nitrogen treatments, and species and nitrate-nitrogen treatments are ,however, very significant. This indicates that the responses of amino acid concentration to these different treatments are essentially different in these 4 different species.

Effects of Solutions of Different Nitrate and Phosphate Concentrations on α-Amino Acid Content

This experiment, like the previous one, was described in detail in Chapter 3. The same experimental species were used: Lolium perenne, Dactylis glomerata, Festuca rubra and Agrostis tenuis. There were 16 nutrient treatments consisting of 4 levels of phosphorus at 4 levels of nitrate-nitrogen in a 4 x 4 grid. The levels of NO_3-N were 0, 1.6, 8, and 32 me./l. The levels of phosphate were 0, 0.48, 2.4 and 12.12 me./l. The concentrations of the other macronutrient ions were the same in each of the treatments with the exception of sulphate. The exact concentrations of the macronutrient and micronutrient ions as well as the nitrogen and phosphorus concentrations of each of the treatments was presented in Chapter 3. The details of amino acid analysis were given in this chapter.

Results

The amino acid response curves of this experiment are presented in the same way as the soluble sugar response curves, that is, in the form of 3-dimensional diagrams. These data are also presented graphically in the form of the response of amino acids to nitrate level at each phosphorus level separately, so that the data may be compared more easily with the results from

the other experiments. The results from this experiment were not analysed statistically because a number of replicates were not included in the final results. The large number of samples in this experiment meant a delay in handling some of the samples. If not all the ethanol extracts produced during the course of a day could be analysed on that day, the samples were stored in a refrigerator in test tubes covered with paraffin wax seals. If amino acid analysis could not be carried out on these samples within one week, the results for such samples were not included in the final results. Tests with glutamic acid standards showed that the amino acid content measured by the method used in this study did not vary significantly over the course of a week.

The effects of nitrate on amino acid at each phosphorus level will be discussed first (Figures 4.3 a-d). The concentrations of amino acids in the 0 me./l phosphorus treatments of L. perenne showed an initial increase with increasing nitrate-nitrogen concentration up to 8 me./l NO_3. Above this nitrogen level, amino acids tended to decrease. The three remaining species showed an initial decrease with increasing nitrate concentration. In F. rubra the amino acid concentration decrease with increasing NO_3 continued to the 8 me./l nitrate level. Above this nitrogen concentration, there

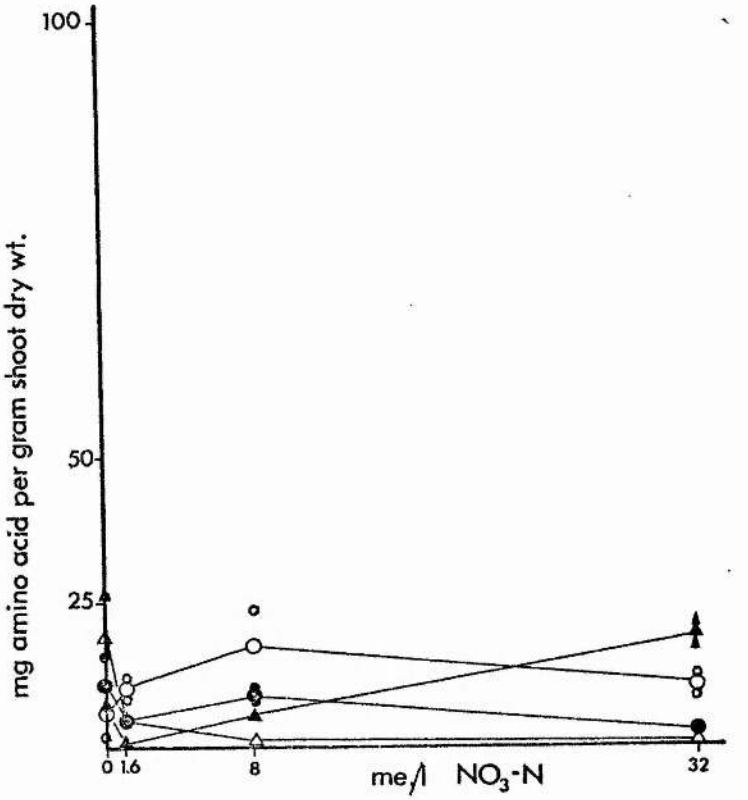


Figure 4.3a: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the amino acid content of 4 species. The amount of phosphorus in each treatment was 0 me./l. Amino acid content was measured in terms of mg amino acid per gram shoot dry weight using a glutamic acid standard. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

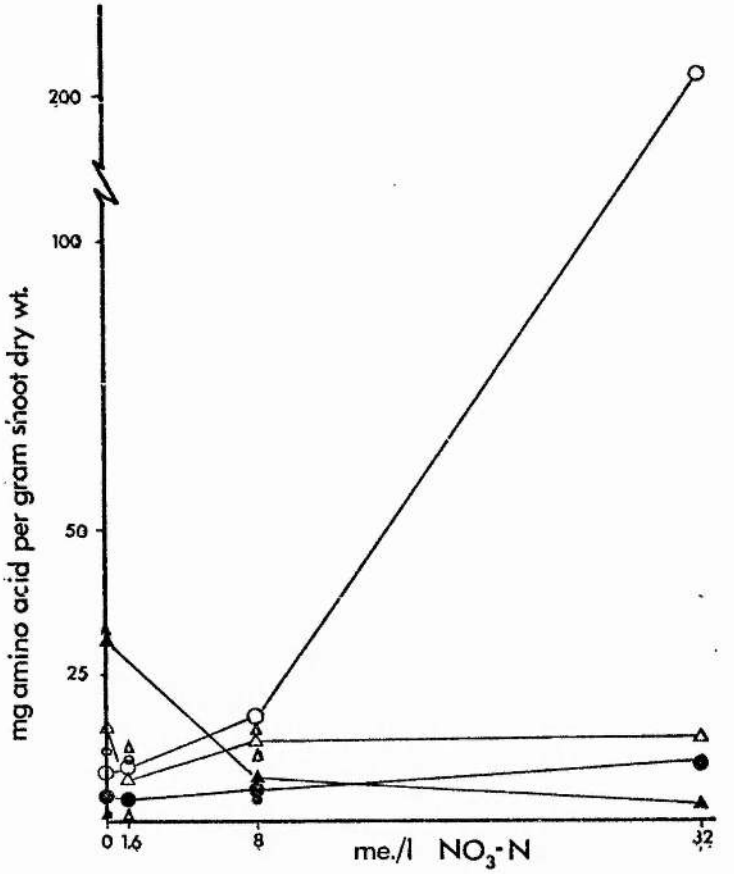


Figure 4.3b: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the amino acid content of 4 species. The amount of phosphorus in each treatment was 0.48 me./l. Amino acid content was measured in terms of mg amino acid per gram shoot dry weight using a glutamic acid standard. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○ = L. perenne ● = D. glomerata
▲ = A. tenuis △ = F. rubra

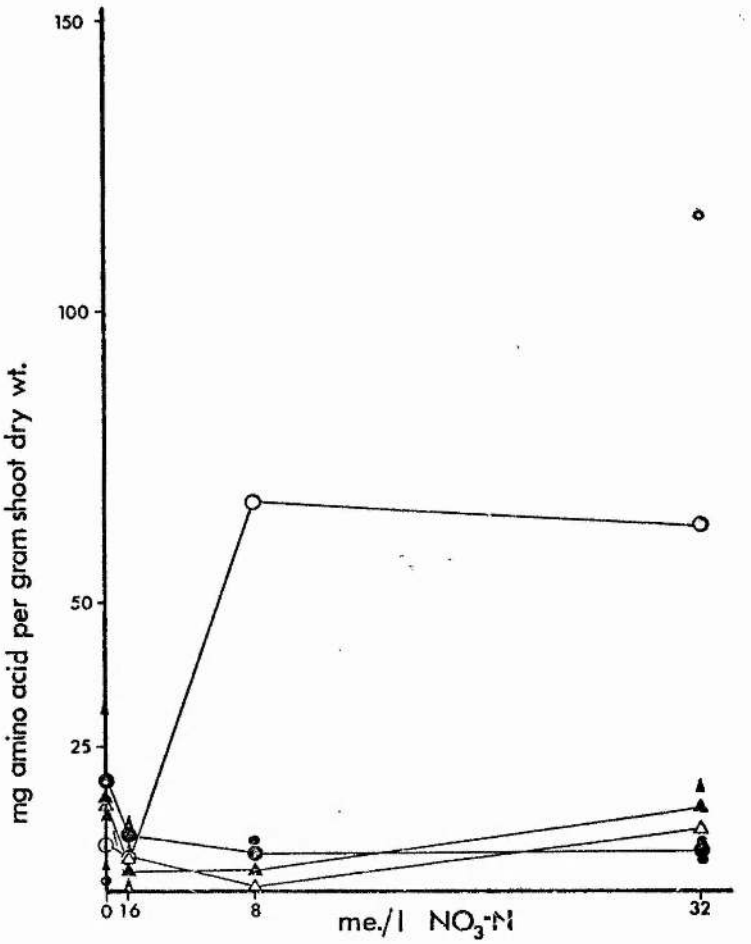


Figure 4.3c: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the amino acid content of 4 species. The amount of phosphorus in each treatment was 2.4 me./l. Amino acid content was measured in terms of mg amino acid per gram shoot dry weight using a glutamic acid standard. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

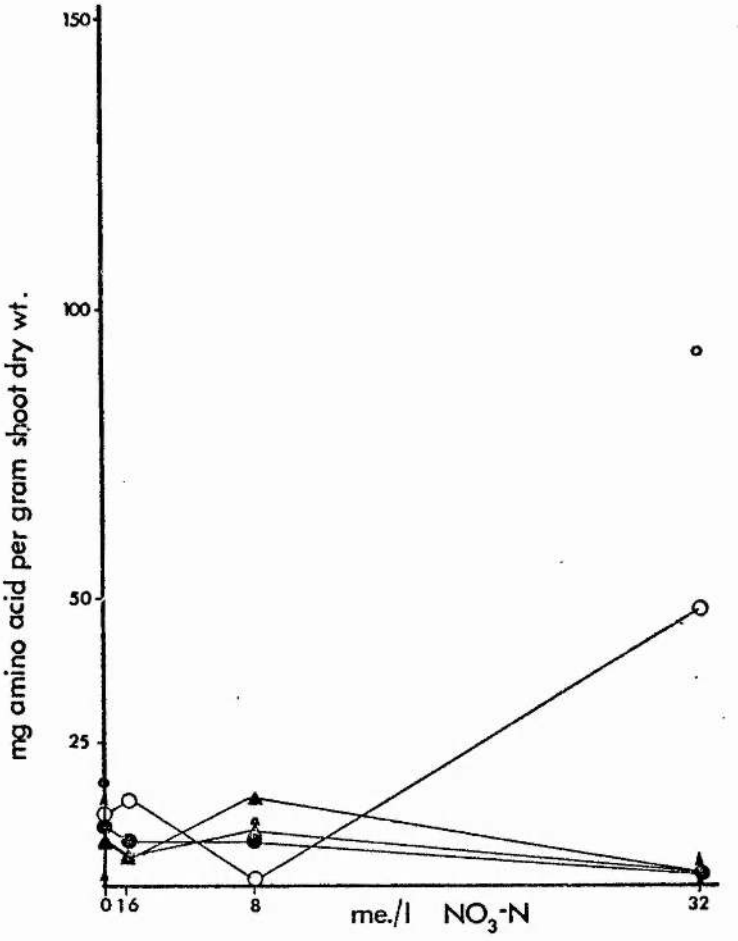


Figure 4.3d: Effect of 0, 1.6, 8 and 32 me./l NO_3-N on the amino acid content of 4 species. The amount of phosphorus in each treatment was 12.12 me./l. Amino acid content was measured in terms of mg amino acid per gram shoot dry weight using a glutamic acid standard. The means of the two replicates are connected by lines and the actual values of the replicates are shown.

○=L. perenne ●=D. glomerata
▲=A. tenuis △=F. rubra

was only a very slight accumulation of amino acids as the 32 me./l nitrate level was approached. F. rubra showed the lowest amino acid concentrations over most of this nitrogen range. A. tenuis showed a continuous accumulation of amino acids with increasing NO_3 concentration from the 8 me./l to the 32 me./l level. D. glomerata only showed this accumulation between the 4 and the 8 me./l NO_3 levels. Above the 8 me./l treatment, the amino acids in this species decreased in concentration. The amino acid response curves are not very different in the 0.48 me./l phosphorus series. L. perenne showed an increase in amino acid concentration with increasing NO_3 up to the 8 me./l nitrate level. This amino acid response was very similar in pattern and concentration to that seen in the 0 me./l phosphorus series but above 8 me./l NO_3, there was a very great accumulation of amino acids. There was only one value for L. perenne in this particular treatment, so, unfortunately, little confidence can be placed in the actual value of amino acid concentration in this treatment. A. tenuis showed a general decrease with increasing nitrate from the lowest to the highest NO_3 level. There was no value in the final results for the 1.6 me./l treatment in this species. With the exception of a relatively high amino acid concentration at 0 me./l nitrate, the

concentrations of amino acids in A. tenuis in treatments containing 0.48 me./l phosphate were lower than those in the series of treatments containing 0 me./l PO_4. F. rubra showed in this series of treatments, an initial decrease in amino acid concentration with increasing NO_3 between 0 and 1.6 me./l NO_3. Above this nitrate concentration, amino acids accumulated to 15.4 mg per gram shoot dry weight. As nitrogen was increased further, this level of amino acid concentration was maintained with only a barely noticible decrease as 32 me./l NO_3 was approached. Lastly, the response of amino acids in D. glomerata to increasing NO_3 was not very different from that seen in the 0 me./l PO_4 series of treatments. There was an initial, albeit slight, decrease in amino acid concentration from 0 to 4 me./l NO_3. This was followed by a continuous accumulation of amino acids as the concentration of nitrate in the treatments increased. When 2.4 me./l PO_4 was included in each treatment, all 4 species showed an initial decrease in amino acid concentration with increasing nitrate level from 0 to 1.6 me./l. L. perenne then showed an accumulation of amino acids to 68 mg per gram shoot dry weight in the 8 me./l NO_3 treatment, an increase of about 62 mg amino acid. Further increase in nitrate concentration resulted in a decrease to 65 mg amino acid per gram

shoot dry weight in the 32 me./l NO_3 treatment. The remaining three species showed very similar amino acid response curves. These consisted of a continued decrease in amino acid concentration to the 8 me./l NO_3 level. In treatments with concentrations of nitrate above this, amino acids in these species tended to accumulate.

When each treatment contains 12.12 me./l PO_4 the amino acid response curves again showed minor differences. Values for the treatments containing 0 and 32 me./l NO_3 were not presented in the case of F. rubra, but over the range of 1.6 me./l to 8 me./l nitrate, amino acids showed an increase in concentration. D. glomerata and A. tenuis both showed an initial decrease in amino acids. This was followed by a general trend for decrease in concentration of amino acids with increasing nitrate concentration. L. perenne again was quite different in amino acid response, and showed an initial accumulation of amino acids followed by a decrease in their concentration seen in the 8 me./l NO_3 treatment. Above this nitrate concentration, the amino acids showed a marked accumulation.

By way of summary, these results for each species will be discussed separately. The responses of the

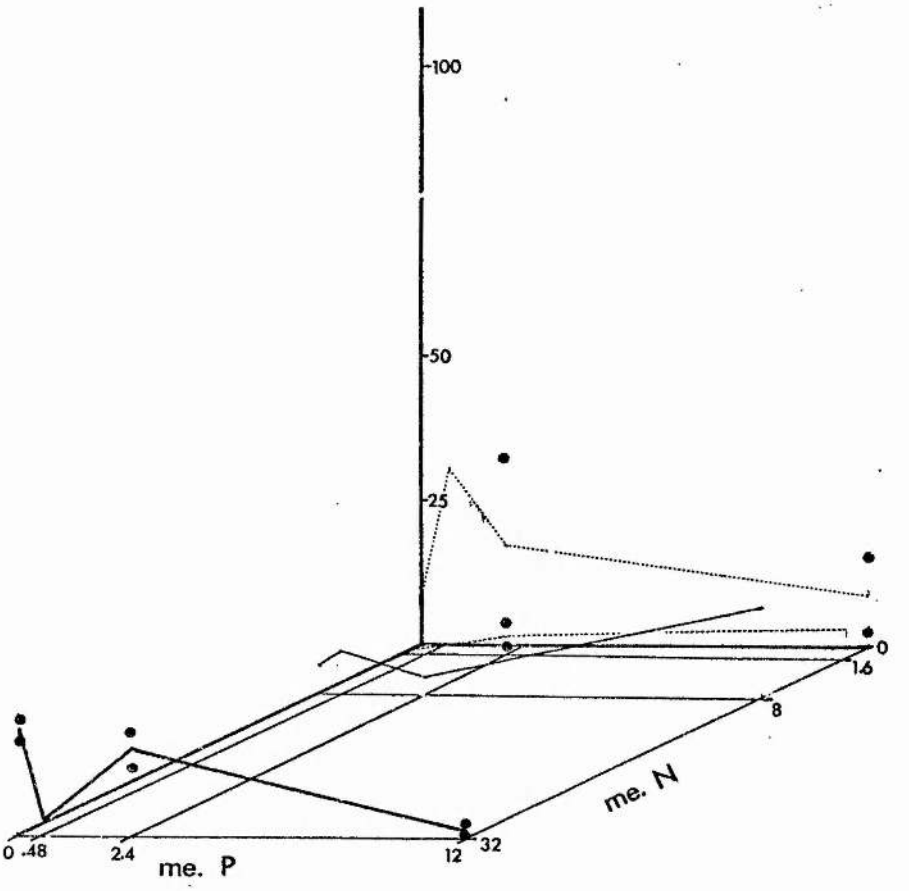


Figure 4.4a: 3-dimensional diagram illustrating the effect on amino acid concentration of different amounts of phosphorus at 4 levels of NO_3-N in A. tenuis. The vertical axis is mg amino acid per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

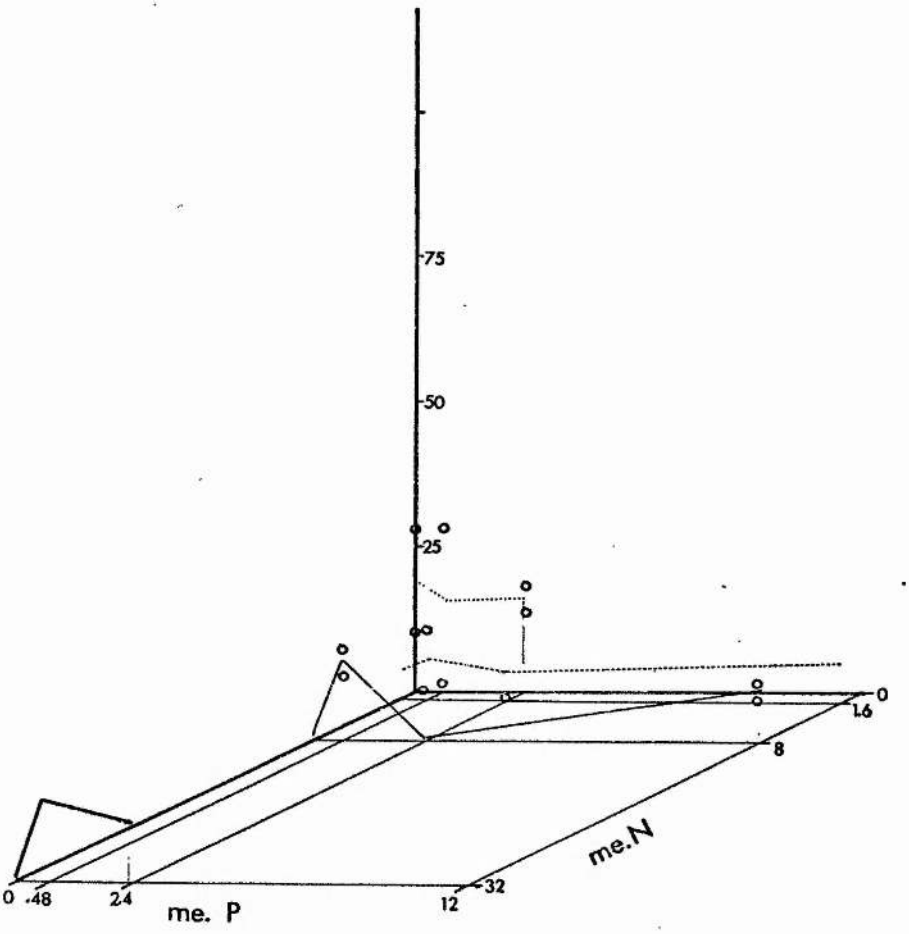


Figure 4.4b: 3-dimensional diagram illustrating the effect on amino acid content of different amounts of phosphorus at 4 levels of NO_3-N in F. rubra. The vertical axis is mg amino acid per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

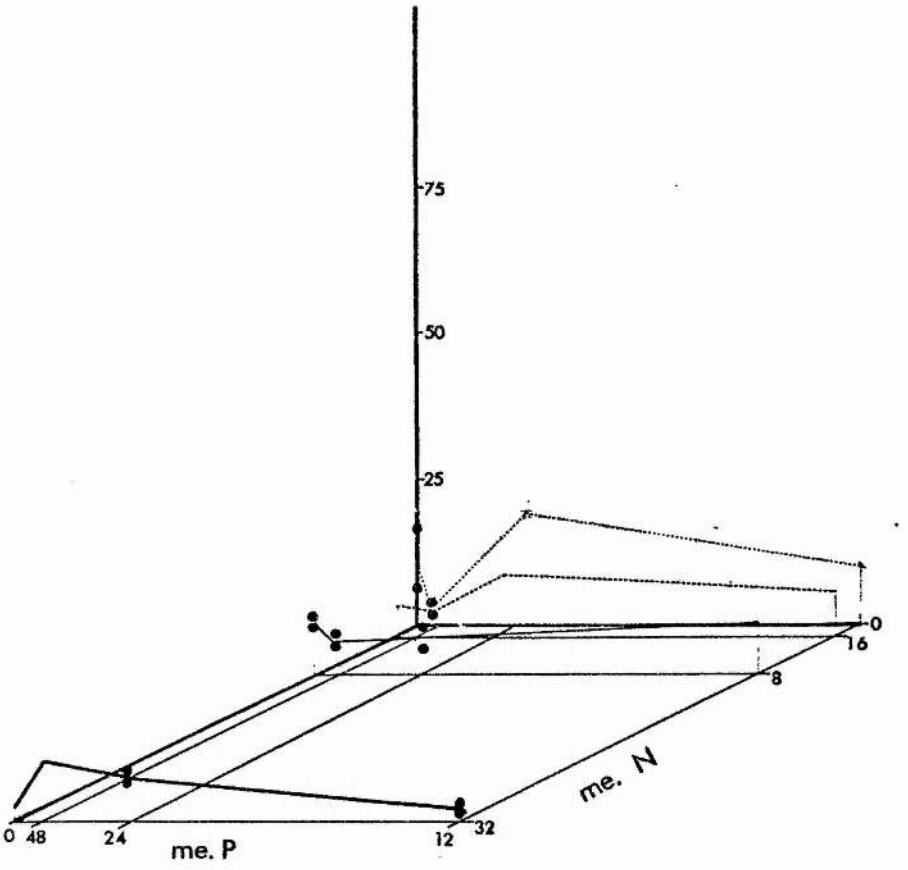


Figure 4.4c: 3-dimensional diagram illustrating the effect on amino acid content of different amounts of phosphorus at 4 levels of NO_3-N in D. glomerata. The vertical axis is mg amino acid per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

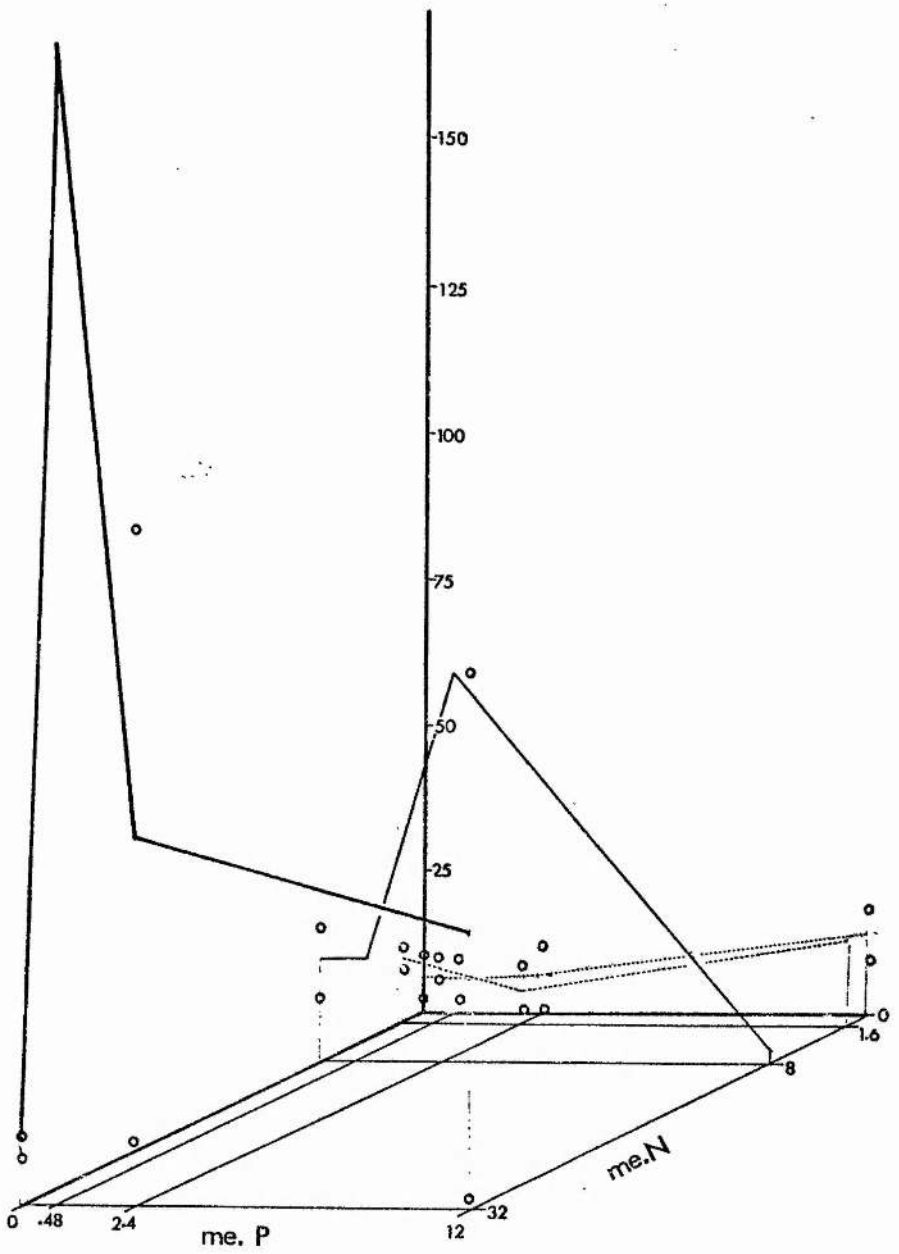


Figure 4.4d: 3-dimensional diagram illustrating the effect on amino acid concentration of different amounts of phosphorus at 4 levels of NO_3-N in L. perenne. The vertical axis is mg amino acid per gram shoot dry weight. The means of the two replicates are connected by lines, and the actual values of the replicates are shown.

amino acids of A. tenuis to increasing nitrate did not change markedly in pattern or concentration as the phosphorus level in the treatments was increased. The amino acids showed a pattern of decrease in concentration with increasing NO_3 level to the 8 me./l NO_3 treatment, followed by an increase in amino acid concentration above this nitrate level in the 0 me./l phosphate and 2.4 me./l phosphate series. In the 0.48 me./l PO_4 series, the amino acids showed a decrease between 8 and 32 me./l NO_3. When 12.12 me./l PO_4 was present in the treatments, the initial amino acid concentration decrease occurred in the 1.6 me./l NO_3 treatment. This was followed by an accumulation of amino acids in the 8 me./l NO_3 level. Additional nitrate resulted in a decrease in amino acid concentration. D. glomerata also showed little difference in the response curves of amino acids to nitrate level in the different phosphate series. Like A. tenuis, there was an initial decrease in amino acid concentration with increasing nitrate in all the phosphate series. In the series containing 0 and 12.12 me./l PO_4, this decrease seen in the 1.6 me./l NO_3 treatment was followed by an increase in amino acid concentration. Further increase in nitrate in these series resulted in a decrease in amino acid concentration in this species. In the 0.48 me./l PO_4

series, the initial decrease in amino acid concentration continued to the 32 me./l NO level. In the 2.4 me./l PO_4 series, the decrease in amino acid concentration continued to the 8 me./l NO_3 level, and further additions of NO_3 resulted in amino acid accumulation.

F. rubra showed a continuous decrease in amino acid concentration with increasing nitrate-nitrogen in the 0 me./l PO_4 series. The addition of 0.48 me./l PO_4 to the nitrate treatments changed the amino acid response curve to one of initial decrease in amino acid concentration to the 1.6 me./l NO_3 level followed by an accumulation of amino acids as 8me./l NO_3 was reached. Further additions of nitrate did not result in much change in the concentration of total amino acids. When 2.4 me./l PO_4 was present in the samples, the amino acids of F. rubra showed a decrease with increasing nitrate to the 8 me./l NO_3 level. Additional nitrate resulted in an accumulation of amino acids.

L. perenne showed the greatest variation in amino acid concentrations between the different phosphate series. This species showed an initial increase in amino acid concentration with increasing nitrate to the 8 me./l NO_3 level in both the 0 and 0.48 me./l PO_4 series. When no phosphorus was present in the treatments, increasing the nitrate level to 32 me./l

resulted in a decrease in amino acid concentration, but when 0.48 me./l PO_4 is present in each treatment, an increase of nitrate to 32 me./l resulted in a great accumulation of amino acids.

Discussion

The metabolism of nitrogen cannot be separated from carbohydrate metabolism or from the growth of the plant. The synthesis of amino acids and proteins is intimately associated with growth, since amino acids are precursors of hormones (eg. indoleacetic acid), coenzymes and pigments (Lehninger, 1975). As well as being the building blocks of proteins, many other biomolecules having specialised functions have amino acids as their precursors.

Because of the importance of amino acids, several studies have been carried out examining the effects of nitrogen nutrition on amino acid content in several species. Margolis (1960) found that ammonium nutrition was associated with an increase in soluble nitrogen compounds in tomatoes. He noted that a marked decrease in soluble nitrogen compounds was the immediate consequence of nitrogen deficiency. It was also found that by supplying nitrate to plants previously deprived of nitrogen, there resulted a temporary increase in basic amino acids and a decline in glutamic acid. This

is particularly interesting, since Margolis' work showed that ammonium nutrition was correlated with high amide values, while nitrate nutrition was correlated with high levels of dicarboxolic acids and glutamic acid. This was explained by correlating a few observations. Under deficiency of nitrogen, soluble nitrogen declined in the leaves of tomato, but asparagine increased in concentration. It was possible that during conditions of nitrogen deficiency, less complex nitrogenous constituents may draw upon protein as a nitrogen reservoir and accumulate as asparagine. When nitrate is again supplied, asparagine decreased in concentration and basic amino acids accumulated. This temporary accumulation preceded the formation of glutamine which may be more conducive to the production of proteins and nucleic acids. Once an adequate supply of nitrate is present, the normal sequence of synthetic reactions occur again, and the accumulated basic amino acids may be rapidly used to synthesise proteins. Although the individual soluble nitrogen compounds were not examined in the present study, this brief discussion was included to present some possible ways in which the observed patterns arose. It appears then, that on the basis of this example, ammonium nutrition is likely to lead to an increase in soluble amino acids. A deficiency in nitrogen should lead to a

decrease in soluble nitrogen compounds in the leaves. Subsequent addition of nitrate should result in an accumulation of total amino acids, as measured by the ninhydrin reaction, although the relative proportions of the different soluble nitrogen fractions may vary. The rapid synthesis of proteins which may follow the renewal of an adequate supply of nitrate may then rapidly use the previously accumulated supply of basic amino acids.

Nowakowski and Cunningham (1966) found that in Italian ryegrass supplied with nitrate, amide-N, glutamine-N and amino-N were lower in concentration than in grass supplied with ammonium-nitrogen, while total nitrogen, total soluble nitrogen and protein-nitrogen were greater in concentration. Kirkby (1968) found that plants supplied with ammonium-nitrogen tended to have increased concentrations of amino acids but lower amounts of non-volatile organic acids. However, Hewitt and Smith (1974) stated that practically all deficiencies of elements causes an increase in amino-nitrogen compounds. Amino acids are built up into proteins if adequate carbohydrate skeletons and energy are present. An accumulation of amino acids could be due to a deficiency of carbon skeletons resulting from the more rapid assimilation of ammonium or as a result of the

removal of excess ammonium from the system. Any factor (eg. low light levels) which would slow the synthesis of protein in the presence of adequate supplies of nitrogen would lead to an accumulation of soluble organic nitrogen, mainly α-amino acids, amides and other simpler nitrogenous metabolites. Nowakowski and Cunningham (1966) found that in Italian ryegrass supplied with ammonuim-nitrogen, there existed an inverse relationship between protein-N and soluble organic-N as a percentage of total nitrogen. When this same species was supplied with nitrate-nitrogen, protein-N was not found to be related to soluble organic-N. However, a linear relationship was found if protein-N (as a percentage of total non-nitrate-N) was plotted against soluble organic-N (also as a percentage of non-nitrate-N). In general, grass given NO_3-N contained much less α-amino-N than grass given NH_4-N. Unfortunately in the present study, the anaylsis of the results of the amino acid measurements was hampered by the lack of statistical analysis in some cases, and the results can only be discussed in the most general terms.

Rapid growth and high yields require the production of much protein, enzymes and other nitrogenous compounds. This in turn requires the protein building blocks, amino acids, which result from

the assimilation of ammonium and nitrate ions and carbon skeletons provided by the soluble sugars, especially the reducing sugars which are the more readily available forms. It is hardly surprising, then, that L. perenne and D. glomerata showed, along with their high yields, a decrease in amino acids and soluble sugar concentrations with increasing concentrations of Hoagland's solution. Although protein content was not measured, it would be reasonable to expect that the protein content would be rising at the same time as the soluble sugars and amino acids were decreasing. When only nitrate varied in the treatments, these species showed an initial increase in amino acid concentration and then a levelling-off effect, again indicating continuous use of amino acids in the face of high yields. When only ammonium varied in the treatments, both these species showed an initial decrease in amino acid concentration followed by a general increase in concentration, especially in L. perenne. It is possible that the more rapid assimilation of ammonium may account for this initial apparent decrease; the level of 4 me./l NH_4-N may be sufficient for a concentration of amino acids to be built up, and once an adequate supply of nitrogen is presented, rapid protein synthesis then uses up the available supply.

The lower yields of the species of oligotrophic environments may account for the patterns seen in these experiments. The responses of A. tenuis and D. glomerata are more erratic than those of the other species and may sometimes be accounted for only in the light of the soluble sugar, especially reducing sugar, content in the same sample. Such discussion will be left for the final chapter. Some patterns may be noted, though. A. tenuis quite often showed very high or, as in the cases of the complete nutrient solution experiment and those dealing with nitrate as the variable ion and different proportions of both nutrient ions, the highest concentration of amino acids per gram shoot dry weight. This may be associated directly with the low yield of this species. In spite of the low amount of growth, there were large quantities of amino acids present, indicating that it was not lack of the essential "building blocks" which held back the growth of this species. Perhaps some amino acids may act as nitrogen storage pools allowing limited growth even under conditions of severe nutrient stress, if the plant makes use of the amino acids produced when conditions were more favourable. As previously stated, these are but a few ideas associated with this chapter, as many of the observed amino acid responses must be discussed with those of the soluble sugars. The

following chapter will summarise the main results of the preceding chapters and discuss further the amino acid responses of these species.

CHAPTER 5

FURTHER DISCUSSION: INTERRELATIONSHIPS OF GROWTH AND SOLUBLE CARBOHYDRATE AND AMINO ACID CONTENTS

It is generally recognised that to obtain maximum production of dry matter and protein from forage crops, heavy fertilisation is necessary, and that species which are widely grown for food generally respond to increased levels of fertilisation with an increase in yield. This situation is the result of the selective breeding of these species for many generations of man, and yet many plants in the wild respond similarly to high nutrient levels. As long as such plants are grown in conditions of high nutrient availability, be they natural or artificial conditions, the rapid response of these plants to nutrient supply may give them a competitive advantage over other slower-growing species in these same sites. However, many plants in the wild do not show these rapid growth rates, and on sites low in available nutrients these species may have the competitive advantage over the faster growing species (Montgomery, 1912).

A number of studies have been concerned with the relative growth rates of various plants and their R-max (maximum potential relative growth rate measured under conditions where no external factor is limiting), and

these have shown that R-max is different in species originating from different habitats (Grime and Hunt, 1975). These studies have shown a number of different patterns:

(1) High levels of R-max are associated with sites of high productivity.

(2) Low R-max is associated with low productivity even on sites of high potential.

(3) In unproductive or stressful situations, low R-max actually seems to be an advantage, while

(4) high productivity in such circumstances seems to be disadvantageous (furthermore, selectively disadvantageous).

(5) There may be a sensitive adjustment of general levels of R-max prevailing in a community in response to variation in site fertility.

These could be confirmed if it could be demonstrated that R-max was of positive or negative adaptive significance and/or its importance is shown by demonstrating that R-max is linked to some other characteristic or characteristics which influence the fitness of the plant in the field.

We must think along these lines in order to explain why there is a low incidence of species with a high growth rate potential on unproductive sites, and why nutrient deficient sites tend to be colonised by

species with low growth rates. Are genetic characteristics which are conducive to rapid growth disadvantageous when environmental extremes are encountered? To answer this, the following observations can be made:

(1) Slower growing plants tend to make modest demands on the environment.

(2) The build-up of reserves in the plant may be allowed due to lower rates of photosynthate and mineral nutrient incorporation into plant structures.

(3) Slower growing species may be better suited to survive conditions where growth is impossible. If some environmental factor arrests growth, a low R-max may prevent rapid deterioration of the plant.

The opposite case, that of the low incidence of plants of low growth rate on highly productive sites, indicates a low competitive ability. There is also a low incidence of these species on disturbed sites (Grime and Hunt, 1975).

It is quite clear that a large number of factors contribute to these observed differences in growth and distribution between species. Are plants adapted to their respective nutrient levels? If the answer is yes, then performance is directly related to fertiliser response, and response is directly related to the plant's ability to exploit the nutrients. There are

many studies which have dealt with the response, as measured in terms of growth, of different species to nutrient supply. Some of these have already been discussed in the text.

In this study, four species of grass were chosen which would be expected to conform to the patterns of growth expected of plants adapted to low nutrient and high nutrient sites. Dactylis glomerata, Lolium perenne, Agrostis tenuis and Festuca rubra were used. L. perenne and D. glomerata were chosen as plants adapted to eutrophic sites. A. tenuis and F. rubra represented those adapted to oligotrophic semi-natural grasslands.

These species were grown in different concentrations of a modified Hoagland's solution for periods of 6 weeks in one experiment in treatments containing 0.1%, 10%, 50% and 100% concentrations of the standard solution, and for 5 weeks in treatments containing 0.1%, 10%, 31%, 68%, 100%, 150%, 200%, 250% and 300% of the same nutrient solution. It was found that in terms of shoot dry weight, F. rubra and A. tenuis showed considerably lower yield than either L. perenne or D. glomerata. If ranked in order of yield, lowest to highest, the order was A. tenuis, F. rubra, D. glomerata and L. perenne. It was apparent from the analysis that the 4 species had similar ranges of yield

under the conditions of these experiments, but their yields at optimum were limited by differences in absolute growth. It will be remembered that because of the different factors contributing to yield in these species, the data were handled in a number of different ways. This study has shown that there existed differences in rates of dry matter production of the 4 species under examination, and that these differences were maintained at different levels of fertilisation. It was seen that the responses of the 4 species to nutrient increase were significantly different. When the data were broken down, it was seen that the species could be separated into two groups on the basis of the levels of significance. On the basis of yield response to increasing nutrient supply, A. tenuis and F. rubra were not significantly different, and L. perenne and D. glomerata were not significantly different. Thus, the separation of the 4 species into two groups on the basis of their response to variation in nutrient supply can be justified when response is measured in terms of shoot dry weight, at least under the conditions of these experiments.

Once the growth patterns of these species had been characterised, it was then possible to study some other aspects of the species' responses to nutrient level. Besides performance in growth, there are metabolic

differences between species which can be measured when the species are presented with a range of nutrient levels. These include the relative amounts of soluble reducing and non-reducing sugars and the relative amounts of amino acids. As stated before, the interaction between nitrogen utilisation and carbohydrate metabolism is a key stage in amino acid and protein synthesis (and therefore growth). As a result, the bulk of this study was the measurement of the alcohol-soluble carbohydrate and amino acid content in the experimental species when grown in different nutrient solutions (Chapters 3 and 4, respectively).

The first of these experiments, like those measuring growth, used treatments containing different proportions of complete nutrient solution. All four species showed an accumulation of soluble sugars at low concentrations of nutrient solution. However, with increasing nutrient strength, the species which showed the significantly greater yields, D. glomerata and L. perenne, responded with decreasing soluble sugar concentration in their shoots. A. tenuis and F. rubra, on the other hand, showed a tendency for accumulation of soluble sugars at high nutrient levels. It would seem that this may be related, at least in part, to growth. When grown under a range of levels of complete nutrient solution, species of oligotrophic environments

may be distinguished by their different patterns of soluble sugar accumulation over this range of nutrition. This may give some clues as to the nature of adaptation to low or high nutrient sites, or may simply be an interesting effect of it. High or low levels of soluble sugars may themselves have something to do with the adaptation to oligotrophic and eutrophic environments, in so far as this adaptation results in the respective soluble sugar levels, and they may be involved with the ability to withstand unfavourable conditions.

In an attempt to discover if the observed patterns of soluble sugar response were due to the reactions of the species to all of the nutrient ions present or due to one or two of them, experiments were carried out in which nitrate, ammonium, ammonium and nitrate, or phosphorus varied in concentration while the remaining nutrient ions did not.

It was noted in the discussion following the examination of the effects of the kind and amount of nitrogen on soluble carbohydrate content that, based on the literature, there were several factors which may influence soluble carbohydrate content in higher plants. To reiterate, it appears that factors such as a low amount of growth (providing photosynthesis is maintained), very high rates of fertilisation (probably

causing a depression of growth due to high concentrations of NH_4 or NO_3 ions which, at the same time, do not halt the production of soluble carbohydrates), nitrogen and phosphorus deficiences, and low rates of amino acid/protein synthesis favour high soluble carbohydrate content. On the other hand, growth rates rapid enough to use the immediate products of photosynthesis as they are produced, increased rates of amino acid/protein synthesis, and a rapid assimilation of NH_4, increased nitrogen fertilisation, and decreased photosynthesis have been associated with low concentrations of soluble carbohydrates.

When the responses of the three soluble sugars to different nutrient regimes are examined, it is seen that the relatively uncomplicated patterns of soluble sugar response in the four species when different levels of modified Hoagland's solution were supplied are quite different from those resulting from the use of various levels of one or two nutrient ions. The results of the nitrate-nitrogen/ammonium-nitrogen and the nitrate/phosphate experiments can be compared on the basis of the statistical significance of the sugar response. When only nitrate varied from treatment to treatment, the variability due to treatment, species differences, and species/treatment interaction effects of the reducing sugars was statistically significant.

The same was true when ammonium was the variable ion. In the series in which the NO_3-N:NH_4-N proportions varied from treatment to treatment, the variability due to treatment and species effects was statistically significant, although that due to species/treatment interaction was not. When reducing sugar response to increasing nitrate at different phosphate levels is reviewed, the variability due to treatment, species, and species/treatment interaction effects was seen to be significant in the 0 me./l, 0.48 me./l and 12 me./l treatments with 5%, 1% and 0.1% levels of significance, respectively. The variability in reducing sugar response in the 2.4 me./l phosphate treatments could not be attributed to any of these effects.

The variability found in the response of the non-reducing sugar sucrose was not so consistant in its source. When nitrate varied in the treatments, variability due to treatment, species, and species/treatment interaction effects was significant, as was the case with the reducing sugars. However, when the variable ion was ammonium, only the variability due to treatment effects was significant. When ammonium- and nitrate-nitrogen proportions varied, variability in sucrose response could be attributed to treatment, species, and species/treatment effects; the variability in all cases was statistically significant. With

increasing nitrogen at different phosphate levels, the variability due to treatment effects was only significant in the two lowest phosphate levels - 0 me./l and 0.48 me./l phosphate. Variability due to species differences was only significant in the 0.48, 2.4 and 12 me./l phosphate treatments, and that due to species/treatment interaction was significant only in the 0.48 and 2.4 me./l phosphate treatments. On the basis of the statistical analysis, which is given in more detail in Chapter 3, there is evidence that these four species are affected by the kind and amount of nitrogen supplied to them, alone and in the presence of different amounts of phosphate, as reflected in their soluble sugar content. Further, at least in the cases of reducing and non-reducing sugars when only ammonium ion content varies, and reducing sugars and sucrose when nitrate ion content varies in the presence of different amounts of phosphate, these species respond to the treatments in ways which are essentially different, as indicated by species/treatment interactions.

The soluble sugar and amino acid concentrations in the four experimental species in response to increasing complete nutrient solution, increasing nitrate and ammonium concentrations, and nitrate and phosphate combinations can now be discussed together. The results

of the complete nutrient solution experiment were illustrated in Figure 4.1. In this case, the total amino acids of L. perenne and D. glomerata were seen to be very similar, both in pattern of response and in concentration. In these species, the decrease in amino acids is probably due to the rapid use of the amino acids produced in the very low nutrient levels to build the proteins and other nitrogen-containing macromolecules needed in the growth of the plants. The gradual and small increase in amino acids with further nutrient increase would then represent an accumulation of amino acids above the level required for growth. Indeed, over the nutrient range in which this amino acid increase becomes noticible (ie. 150%-200% modified Hoagland's solution), the relative growth rates in these species are seen to level off and begin to drop. Further, in the preliminary experiment in Chapter 3 examining soluble sugar content in relation to growth in complete nutrient solution, these species tended to show a decrease in soluble sugar content with increasing nutrient concentration. This slowing of growth rate in the very high nutrient levels combined with low soluble carbohydrate levels and an increasing amino acid concentration may indicate that there is a toxicity effect at these high nutrient levels and that photosynthesis becomes restricted. The low amino acid

concentrations of these species in comparison to those of A. tenuis and F. rubra may well be due to the greater yields of L. perenne and D. glomerata and hence greater incorporation of amino acids into proteins, membranes, enzymes, etc.

The fluctuating response of the species of oligotrophic environments, F. rubra and A. tenuis, is more difficult to explain. It is seen, however, that the responses of these species are similar in pattern. These were the species with the lower relative growth rates, lowest yields and the tendency to accumulate soluble sugars when grown in treatments of increasing nutrient strength. It would seem that the higher amino acid concentrations in these species is due to the lack of incorporation of these amino acids into proteins and other nitrogenous compounds characteristic of rapidly expanding plant tissue, accounting also for the relatively lower amount of growth.

When only nitrate was increased, L. perenne and D. glomerata showed the expected increase in amino acid concentration. Both of these species showed minor fluctuations with increasing nitrate, but in general, the amino acid concentration was maintained around 25 mg per gram shoot dry weight, the concentration of amino acids in L. perenne being higher than that of D. glomerata in the higher nitrate levels. A. tenuis and

F. rubra again showed marked fluctuations in amino acid concentration. A. tenuis generally showed higher amino acid concentrations than the other species, while F. rubra showed the lowest levels. The higher concentrations in A. tenuis can be related to the relatively lower levels of reducing sugar concentration (ie. use of carbon skeletons) in this species with increasing nitrate. The sucrose concentration in this species was relatively low at 16 me./l NO_3, and this sugar accumulated when nitrate was increased above this, while below 16 me./l NO_3, the total amino acids were greater in concentration than above this NO_3 level. Thus, the amount of total amino acids appears to be inversely related to the amount of soluble carbohydrates.

The amino acid concentration of the four species grown in treatments of varying ammonium-nitrogen concentration was illustrated in Figure 4.2 b. The general trend of amino acid concentration in the species of oligotrophic environments, A. tenuis and F. rubra, showed a general trend for amino acid accumulation with increasing ammonium-nitrogen. This was consistant with the work of Margolis (1960) and others. These species also showed a marked decrease in amino acid concentration with nitrogen deficiency (in this experiment, 4 me./l NH_4). In this case, amino acid

concentration appears to be inversely related to reducing sugar concentration above 24 me./l NH_4 but positively related to reducing sugar concentration below 24 me./l NH_4. L. perenne and D. glomerata showed an initial decrease in amino acid concentration as NH_4 was increased from 4 to 8 me./l. This may be related to the rapid growth rates of these species and their very high yields. Utilisation of amino acids in these species may be much more rapid than in the species of oligotrophic environments. Certainly in L. perenne the soluble sugars show a dramatic decrease in concentration over this range, indicating their rapid utilisation. The lower amino acid concentrations of D. glomerata follows the pattern of lower reducing sugar concentration in this species. In these two species, with the exception of the amino acid response of L. perenne in the 48 me./l NH_4 treatment, the amino acid content seems to follow their reducing sugar content closely.

When the amino acid content of these species in response to different proportions of ammonium- and nitrate-nitrogen (Figure 4.2 c) was examined, it was seen that the species of oligotrophic environments, A. tenuis, showed the highest concentrations of amino acids of the four species over the entire range of treatments. In this species, the amino acid content of

the plants from the treatment containing 24 me./l NH_4 and no NO_3 was relatively low but about the same value as the 24 me./l NH_4 treatment in the previous section of this experiment. On increasing the NO_3 content to 4 me./l and decreasing the NH_4 content to 20 m./l, amino acid content nearly trebled. Increasing the NO_3 proportion brought about a general decrease in amino acid concentration in this species. All three of the soluble sugars showed this same dramatic accumulation in this high ammonium-nitrogen / low nitrate-nitrogen treatment. There is then no shortage of carbon skeletons, so the great accumulation of amino acids must be due to either lack of utilisation or a surplus of amino acid above that which can be used. The decrease in amino acids following increasing proportions of NO_3 is also reflected in the soluble sugar content. The decreasing sugar content is likely to be due to the increasing use of carbohydrate to reduce NO_3 as well as to assimilate NH_4. No doubt the species' low growth rate influences the quite high levels of soluble sugars and amino acids.

There is no great difference in pattern of amino acid response or level of concentration between the other species. In L. perenne and D. glomerata, the greatest concentration of amino acids occurred in the treatments containing 8 me./l NO_3-N and 16 me./l NH_4-N.

The greatest amino acid concentration in F. rubra occurred in the treatment containing 12 me./l of both nitrogen ions. In all three species, when the concentration of either ion was increased above that in their respective optimum amino acid response treatments, amino acid content decreased. There was a greater decrease when the proportion of nitrate was increased. It is notable that the patterns of soluble sugar response and content (especially that of the reducing sugars) are different in A. tenuis and the group formed by the other three species. The higher amino acid content in the treatments containing higher proportions of ammonium-nitrogen was coincident with the high concentrations of reducing sugars. The decrease in reducing sugar concentration with increasing NO_3 proportion from 0 me./l and the increase in amino acid concentration may indicate the assimilation of nitrogen ions.

The three parts of this experiment were combined and when they were examined statistically, it was found that changing proportions of the two nitrogen ions had a statistically significant effect on the amino acid content in the species. The variance due to NO_3-N was significant to the 5% level, while that due to NH_4-N was highly significant at the 0.1% level. It will be remembered that when the same analysis was carried out

on the soluble sugar response in the same experiment, the effect of nitrate-nitrogen was highly significant on both reducing sugars, while that of ammonium-nitrogen was not significant. The effect of NO_3-N on sucrose was not significant, while that of NH_4-N on sucrose was highly significant. Additionally, the variability due to species differences was statistically significant in the response of the reducing sugars but not in the response of sucrose. While the amino acid responses showed significant species/NO_3-N and species/NH_4-N effects, none of the soluble sugars examined showed significant interaction effects. The differences between the species in the response of their soluble sugar contents to the kind and quantity of nitrogen ions appear to be primarily quantitative differences. However, the significance of the interaction effects in the amino acid responses of these species may indicate that the way in which amino acids respond to these treatments is essentially different in the different species, and that when investigating the adaptation which some plants apparently have for low nutrient sites, it is the fates of the amino acids which should be studied in close detail (at least where nitrogen nutrition is concerned).

To review the species in the light of this phase

of the discussion, the species of eutrophic environments, L. perenne and b. glomerata, were those which showed high relative growth rates and very high yields compared to the other species. When nitrate was the only variable ion in the treatments, amino acid concentration was roughly inversely proportional to reducing sugar concentration. Though showing fluctuations with increasing NO_3-N, the amino acid content in these species was fairly constant at high levels. In view of the high yields of these species, and their apparent adaptation to the higher nutrient sites, this seems to indicate a constant utilisation of the carbohydrates produced in photosynthesis as well as the utilisation of the amino acids produced. When ammonium-nitrogen is the variable ion in the treatments, these species showed an initial decrease in amino acid content with increasing NH_4-N. This may be related, again, to the rapid growth and high yields in these species and especially to more rapid assimilation of NH_4-N. Certainly L. perenne showed a rapid decrease of soluble sugar with increasing NH_4-N, and this can be related to the use of the carbohydrate skeletons. Further, when different proportions of the nitrogen ions are supplied, amino acid concentration is directly proportional to reducing sugar concentration.

The species of oligotrophic environments, A.

tenuis and F. rubra, showed very low yields when measured in terms of shoot dry weight. Although F. rubra did not show responses very like those of A. tenuis when grown in solutions differing in the kind and amounts of nitrogen ions, A. tenuis showed some clear differences from L. perenne and D. glomerata. In the majority of treatments, A. tenuis showed relatively high levels of amino acid concentration, and in the treatments in which only NO_3-N and those in which different proportions of the two nitrogen ions varied, A. tenuis showed the highest levels of amino acids. In this species, when only NO_3-N varied, the shoots contained relatively low levels of reducing sugars, and these decreased in concentration with increasing NO_3-N. This pattern may indicate a low use of amino acids produced from the products of photosynthesis. Both species showed an accumulation of amino acids with increasing NH_4-N and a sharp decrease in amino acid concentration with ammonium deficiency. These species also showed a sharp decrease in reducing sugar concentration with ammonium deficiency. In the treatments containing both ions, the levels of reducing sugars and especially amino acids were high compared to the other species in the highest ammonium treatment. This was followed in the next treatment by a very great accumulation of amino acids and soluble sugars.

It may be that under conditions of ammonium nutrition, the two species adapted to low nutrient sites accumulate reducing sugars and amino acids because of their low yields and rates of growth, or due to lack of utilisation of amino acids produced.

When examining the results of the experiment which studied the amino acid response to both nitrate and phosphate, the results can most easily be compared to the previous experiments using the illustrations of the effect of NO_3 at each phosphate level (Figures 4.3 a-d). Again, the amino acid concentrations in these species are more related to the reducing sugar concentrations of the species than to the concentration of sucrose. In cases of phosphorus deficiency, sucrose and reducing sugars can accumulate (Hewitt and Smith, 1974), while proteins and nucleic acids decrease in concentration, but amides which need ATP for synthesis and amino acids tend to increase in concentration as proteins are broken down.

In this experiment, many of the patterns observed in the different species were similar. Examining them separately, A. tenuis was seen to decrease initially in amino acid concentration with increasing nitrate when 0 me./l phosphate was present, and then increase with increasing nitrate level. When 0.48 me./l phosphate was present there was a continuous decrease in amino acid

concentration. When 2.4 me./l P was present, this decrease gave way to accumulation when amounts of NO_3 greater than 8 me./l were supplied. In the highest concentration of phosphate, after an initial decrease in amino acid concentration, amino acids accumulated in the 8 me./l NO_3 treatment before decreasing again at higher nitrate levels. The other species of oligotrophic environments, F. rubra, had a similar pattern and level of concentration of amino acids to A. tenuis in the two higher phosphate levels, though in the 0 me./l PO_4 series it showed a continuous decrease in amino acid concentration and in the 0.48 me./l PO_4 series showed some amino acid accumulation at the 8 me./l NO_3 level. Unlike the previous experiments in which A. tenuis appeared to have much greater amino acid concentrations than the other species, these species showed relatively low amino acid levels. The amino acid concentration could not, therefore, be easily related to the low yields of this species.

L. perenne and D. glomerata were similar in their responses to the 0 me./l PO_4 series, showing very similar patterns of reducing sugar response in nearly all the phosphate concentrations. This may indicate that these species differ not in production of amino acids, but in their use to synthesise proteins, etc. The pattern in the phosphorus deficient treatments was

one of accumulation up to the 8 me./l NO_3 level, then gradual decrease in amino acid concentration as nitrate was further increased. In the 0.48 me./l PO_4 series, the pattern was one of gradual increase in amino acid concentration in the case of D. glomerata, and a rather dramatic increase in the case of L. perenne. This difference cannot be related to soluble sugar concentration as the patterns and concentrations in these two species were quite similar. In the next phosphate series (2.4 me./l PO_4), both species again showed the initial decrease in amino acid concentration, but in L. perenne this was followed by an accumulation of amino acids at the 8 me./l NO_3 level, which seems to stabilize at this relatively high concentration. In D. glomerata, amino acid concentration decreased to the 8 me./l NO_3 level and stabilized here, with only a slight accumulation in the 32 me./l NO_3 level. Again, the patterns of soluble sugars are so similar in these species, this difference in amino acid pattern is difficult to account for. In the highest phosphate level, these two species fluctuate exactly out of phase. While L. perenne showed an initial amino acid increase D. glomerata showed an initial decrease, and so on.

In examining the amino acid response to nitrate in this same experiment, the results do not immediately

suggest that the species of oligotrophic and eutrophic environments are essentially different. The only real indication is the generally higher levels of amino acids in L. perenne and a tendency for greater concentrations of amino acids in this species as the phosphate content increases at least to the 2.4 me./l PO_4 level. As stated before, these results are difficult to account for on the basis of the growth experiments described in Chapter 2 and on the response of soluble sugars in the same experiment.

The data for this experiment was also presented in the form of 3-dimensional illustrations, both in terms of soluble sugar response, and amino acid response. Although it is the more readily available reducing sugars which will be related to the amino acid concentrations, it is notable that the expected accumulation of soluble sugars with phosphorus deficiency is far greater in the non-reducing sugar sucrose than in the reducing sugars. This dramatic accumulation of sucrose occurs in F. rubra and A. tenuis at all levels except, of course, that in which nitrate is deficient also. However, in L. perenne and D. glomerata, sucrose actually decreased in concentration as phosphate content was reduced to 0 me./l, also in the highest nitrate level. This tendency for decrease at this level is also seen in the reducing

sugars in these species, perhaps indicating that these species are more susceptible to imbalances of these nutrients.

The patterns of both reducing sugar response in A. tenuis to increasing phosphate were very similar, differing mainly in the much greater concentrations reached by fructose. In pattern of response, the amino acid content of A. tenuis varied directly with reducing sugar response at all nitrate levels except that containing 8 me./l NO_3. The reducing sugars of F. rubra were also very similar in pattern. Again fructose was much greater in concentration, but the glucose pattern at the highest nitrate level was different. Glucose showed an increase in concentration as phosphate increased to 2.4 me./l, and this was followed by a further, albeit small, accumulation. Fructose, on the other hand, showed an initial decrease in concentration, an accumulation as 2.4 me./l PO_4 was reached and, finally, a decrease in concentration. Although some of the values for amino acid content were missing in the case of F. rubra, there were two complete nitrate series, 1.6 and 8 me./l NO_3. When each treatment contained 1.6 me./l NO_3, amino acid concentration varied directly with reducing sugar concentration. As was the case in A. tenuis, the amino acid concentration of the 1.6 me./l NO_3 treatment

varied inversely with reducing sugar concentration.

The reducing sugars of D. glomerata were, again, very similar in pattern, and the concentrations of fructose were much higher than those of glucose. The only major difference was that glucose showed a small increase in concentration in the 2.4 me./l PO_4 treatment relative to the 0.48 me./l PO_4 treatment in the highest nitrate series, while fructose showed a decrease over the same range. When no nitrate was present, the pattern of amino acid response was inversely related to reducing sugar response, and directly related to to reducing sugar response in the 1.6 me./l NO_3 series above 0.48 me./l PO_4. As in the other species in the 8 me./l NO_3 series, amino acid response was inversely related to reducing sugar concentration. At the highest nitrate level, amino acid response in D. glomerata was directly related to reducing sugar concentration below 2.4 me./l PO_4. Again, the two reducing sugar responses were similar in pattern in L. perenne, the other species of eutrophic environments. The main difference was that the fructose concentrations were higher than the glucose concentrations. The amino acid response to phosphorus in the 8 me./l NO_3 series was, as in all the other species, inversely related to reducing sugar concentration. The amino acid response in the 32 me./l

NO_3 series was roughly directly related to reducing sugar response. The amino acid response in the 0 me./l NO_3 treatment was inversely related to reducing sugar response, and that in the 1.6 me./l NO_3 treatment was inversely related to reducing sugar response above 0.48 me./l PO_4.

It is clear from the above discussion that amino acid response to different phosphate and nitrate levels is more easily related to reducing sugar concentration when the data is organised as amino acid response to increasing phosphate and each nitrate level is considered separately. In cases where the phosphate level is variable, this ion may have an overriding effect on soluble sugar and amino acid concentration. As to the differences between the species from oligotrophic and eutrophic environments, in this experiment the most obvious is the higher concentration of amino acids in L. perenne especially in the higher nitrate levels.

Although this examination of the results of all of the experiments is quite lengthy, and in some cases did not appear to clarify the differences between the species of oligotrophic and eutrophic sites, it did explain some of the fluctuations of soluble sugar content in these species. It is clear that such data is worthwhile, and that the differences between these

species has been demonstrated. However, further information would be necessary for a more complete explanation of the phenomenon, information such as protein content, inorganic as well as other organic nitrogen compounds in the shoot, root and seeds, as well as the effects of other ions individually.

To some extent the variation in the responses of the species could be due to reponses of the plants to aspects of the glasshouse environment which could not be controlled or indeed even identified at the time of the experiments, since so little is known about the extent to which plants react to their macro- and microenvironments. As far as possible, the conditions under which the experiments were conducted were controlled and were as identical as possible for each species and treatment, so until these as yet unidentified effects are taken into account, the results of this study must be considered valid. Finally, future investigations in this area would logically be the demonstration of the observed effects in the field.

SUMMARY

The approach in this study to discover more about the relation between growth rate and adaptation to particular sites was an examination of the quantitative differences in growth, soluble carbohydrate and amino acid content in the four experimental species: L. perenne, D. glomerata, A. tenuis and F. rubra.

(1) On the basis of the growth studies, the species of oligotrophic and eutrophic environments could be seen to differ, and form two groups: L. perenne and D. glomerata in one group, and A. tenuis and F. rubra in the other, the species of eutrophic and oligotrophic environments, respectively.

(2) When complete nutrient solutions were supplied to these species in treatments of increasing concentration the two groups were seen to differ in the pattern of response of their soluble carbohydrates. When the treatments used in further experiments differed only in nitrate, ammonium, nitrate/ammonium proportions and phosphate at different nitrate concentration, definite treatment effects were found in each species, and in many cases, the response of the species could be seen to be significantly different. Further, in many cases, the differences divided the species into the same two groups observed in the growth experiments.

(3) However, when the effects of the separate ions were examined, these species differences appeared to be due mainly to quantitative differences rather than to differences in pattern of response.

(4) The examination of amino acid content of the species did not appear to clarify the differences between the species of oligotrophic and eutrophic sites, though this examination did explain some of the fluctuations of soluble sugar content which could in some cases be related to the yields and rates of growth in the species.

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