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The influence of temperature, light intensity and nitrate concentration on dry-matter production and chemical composition of *Lolium perenne L.*

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Summary

In this experiment the nitrate concentration of the nutrient solution influenced the rate of dry-matter production only when it fell below 0.2 mmol. Between this value and a value of ca. 1.5 mmol the chemical composition was considerably altered but production was not influenced. The soluble carbohydrate content, which reached a very high value in the above ground parts when the nitrate supply was low, fell as the nitrate concentration rose to the higher level and the same tendency could be observed in the crude-fibre content and some unknown components, probably hemicelluloses and pentosans. The organic-nitrogen fraction on the other hand, increased with increasing nitrate concentration in the above-mentioned range, whether calculated on a soluble-sugar-free basis or on the basis of total dry weight. Above a concentration of 1.5 mmol the effect was almost exclusively confined to the nitrate concentration in the plant; in this range of values there was always a definite relationship between the nitrate content and the soluble-carbohydrate content.
The optimum temperature for dry-matter production was found to shift to higher values at higher light intensities. Usually the soluble-carbohydrate content decreased with increasing temperature; and with increasing light intensity it always increased. Of the remaining plant constituents only the nitrate content was influenced by temperature and light intensity, for the other plant components analysed remained the same when calculated on a sugar-free dry weight.
This difference between nitrate concentration on the one hand and temperature and light intensity on the other was related to effects on the rate of tillering, which is influenced by the NO_3-concentration, but not by the other factors.

1. Introduction

In previous studies (ALBERDA, 1957, 1960) it was shown that temperature, light intensity and nitrogen nutrition have a distinct influence on the soluble-sugar content of perennial ryegrass. Considering the increasing importance that is becoming attached to a high carbohydrate content in relation to feeding value, silage making and regrowth, it was thought worth while to study the influence of these factors, separately and in combination, under otherwise constant conditions in growth chambers.
In the present study not only the soluble-carbohydrate content is taken into consideration, but also the influence of the above-mentioned factors on dry-matter production and on some other plant constituents.

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2. Materials and methods

All the experiments were carried out in the phytotron of the Institute. The plants were usually cultivated in a temperature-controlled greenhouse at 20° C, but the experiments were carried out in growth rooms with artificial light from high-pressure mercury vapor lamps (HPL 400 W) (ALBERDA, 1958) giving a light intensity at plant height of approximately 5×10^4 ergs cm^{-2} sec^{-1}. Further details are given with the separate experiments.

The plant material consisted of clonal material from a tetraploid perennial-ryegrass strain. Separate tillers of this clone were freed from dead material, and after the roots had been cut back, they were fixed in bunches of seven in holes in a hardboard disc using strips of foam plastic. Discs with four of these holes were placed on top of one-litre glass jars, which were first painted black and then with an aluminium paint. In some experiments 21 of these hardboard discs were placed on top of an earthenware wash stand of 35 litres capacity instead of on glass jars. With the latter set-up it was easy to change the nutrient solution for all 21 sets of plants quickly. In addition each set had more solution at its disposal than on the glass jars, and for this reason the method was especially used in nitrogen-concentration experiments.

All plants were first put on tap water. Under these conditions they rapidly formed new white roots. After a week the tap water was usually replaced by a half strength Hoagland solution, which was renewed twice a week. The solution in the wash stands was continuously aerated to ensure an even distribution of minerals. Aeration by itself had no influence on dry-matter production. During the experimental periods the solution was renewed at least three times a week but very often daily (except Sundays). This will be indicated in the separate experiments.

When the plants were harvested the roots were cut off and the shoots were cut 5 cm above the base, thus dividing them into stubble and leaves. All three portions were washed quickly with tap water, blotted with filter paper, weighed and dried in a forced-draft oven at 70° C for at least two hours, followed by half an hour at 105° C. The material was then weighed again, ground and stored for analysis in well-stoppered bottles.

The chemical analysis was carried out in the dried and ground plant material as follows: –

Total nitrogen

With the normal Kjeldahl method a part of the nitrate nitrogen in the plant tissue may escape as NH_3, and therefore in this method some salicylic acid is added together with sulphuric acid (DEYS, 1961) so that the nitrate is bound as nitrosalicylic acid. This is converted to amino-nitrogen by adding sodium thiosulphate after which the normal Kjeldahl degradation follows.

Nitrate nitrogen

This is determined using the method of DIJKSHOORN and LAMPE (1960) in which the nitrate is converted into nitroxylenol after the sugars and chloride ions had been removed with cuprous sulphate plus calcium oxide and silver sulphate respectively.

Total water-soluble carbohydrate (TSC)

The plant material is extracted with boiling water. To the extract neutral lead acetate is added to remove reducing non-sugars. After filtering the surplus lead acetate is taken away with disodium hydrogen phosphate and the solution is filtered again. The sugars are hydrolyzed with 0.05 N sulphuric acid on a boiling water bath for half an hour and after neutralizing are determined by the VAN DER PLANK (1936) method. These carbohydrates may also be referred to as sugar or soluble sugars in the text and as TSC in the figures.

Crude protein

The plant material is boiled first with diluted hydrochloric acid and then with diluted sodium hydroxide. After filtration the residue is washed, dried at ca. 130° C, weighed, ashed at 700° C and weighed again. The loss in weight is taken as crude protein.

Ash

The material is ashed at 550° C and weighed.

3. Results

Experiment 1

Discs with plants on one-litre pots were divided into eight groups according to the following different conditions: –

a. two light intensities: 2×10^4 ergs cm^{-2} sec^{-1} and 5×10^4 ergs cm^{-2} sec^{-1}.
b. two nitrate concentrations: 0.94 mmol and 3.75 mmol.
c. two temperatures: 15 and 25° C.

From the time that the plants were placed under the experimental conditions the nutrient solutions were changed daily, except on Sundays. Because of this, harvests were always taken in the second half of the week to avoid the possible after-effects of nutrient depletion at the weekend. During the course of the experiment, plants were harvested thrice. For the 25° C plants these were 6, 13 and 25 days after the start of the experiment; for the 15° C plants these times were 7, 14 and 26 days respectively.

The course of the dry-matter production during the experiment is given in FIG. 1a. At a high light intensity neither the temperature nor the nitrate concentration of the culture solution had any effect on dry-matter production. At a low light intensity production was independent of nitrate concentration, but temperature had a very distinct influence, higher values being associated with the lower temperature. In most cases dry-matter production was more or less exponential during the whole experiment; a gradual decrease in the relative production rate was, however, observed under conditions of high temperature and low light intensity. Tiller numbers are presented in FIG. 1b which shows a general resemblance to FIG. 1a, except that at high light intensity tillering was reduced at the lower nitrate value. Since there were no concomitant differences in dry weight, the mean tiller weight was clearly higher under these conditions. The temperature did not affect the rate of tillering, although differences in tiller number had already arisen at the time of the first harvest.

At a low light intensity the reverse occurred. The nitrate concentration had no effect, but the temperature effect was very pronounced. At 25° C tillering came to a standstill.

The distribution of dry matter between roots, stubble and leaves is presented in FIG. 2 for the last harvest; the weights of the three plant portions are given as a percentage of the total dry weight.

FIG. 1. Rate of dry-matter production (a) and of tillering (b) under different external conditions

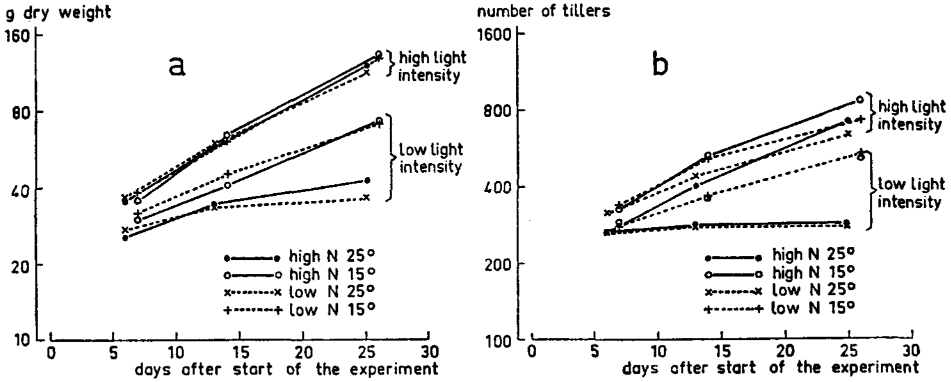
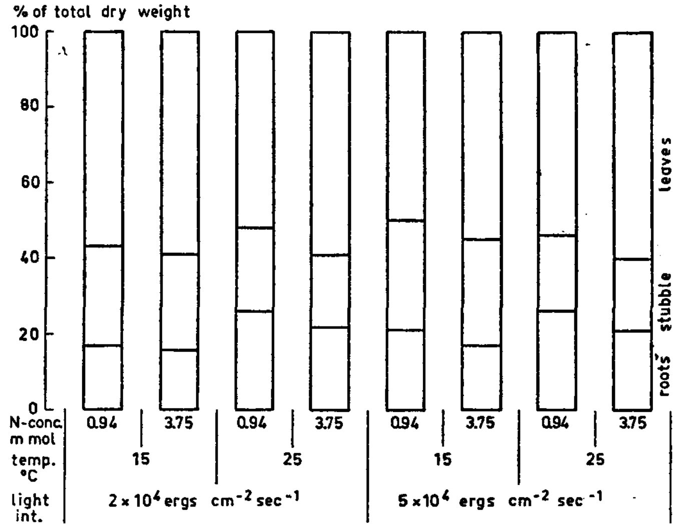


FIG. 2. Distribution of dry matter between roots, stubble and leaves under different external conditions



The most distinct feature of these results is the relative increase in root weight at the low nitrate concentration; this was however much less apparent at 15° C and low light intensity. Another difference in distribution was the low stubble weight at 25° C when compared with 15° C. This was associated with a relatively greater weight of root material and at high light intensity also with more leaves.

The chemical composition of the plants is given in TABLE 1, also for the last harvest. The other harvests show the same features but the trends were slightly less pronounced, especially at the first harvest. The influence of the three different factors will be considered one by one.

The changes brought about by differences in the light intensity principally affected

TABLE 1. Influence of light intensity, temperature and nitrogen concentration on chemical composition

Light intensity:	2×10^4 ergs cm^{-2} sec^{-1}				5×10^4 ergs cm^{-2} sec^{-1}			
Temperature (°C):	15		25		15		25	
N-concentration (mmol):	0.94	3.75	0.94	3.75	0.94	3.75	0.94	3.75
Roots								
NO_3-N	0.45	0.52	0.44	0.49	0.09	0.63	0.05	0.58
Total N	1.88	2.09	1.97	1.95	1.51	2.34	1.59	2.20
T.S.C.	9.0	8.5	4.1	5.0	11.4	7.4	6.5	3.9
Crude fibre	27.1	26.6	28.7	28.7	27.5	28.1	27.7	28.8
Stubble								
NO_3-N	0.20	0.34	0.52	0.76	0.03	0.27	0.05	0.61
Total N	2.11	2.25	2.43	2.63	1.39	2.40	1.54	2.50
T.S.C.	31.5	31.4	14.5	11.6	41.1	34.0	24.6	13.5
Crude fibre	16.6	16.7	24.1	24.4	15.9	15.6	22.8	24.3
Leaves								
NO_3-N	0.18	0.36	0.60	0.89	0.02	0.30	0.02	0.84
Total N	2.94	3.06	3.01	3.38	2.13	3.30	2.25	3.46
T.S.C.	16.0	16.4	4.1	5.0	28.3	19.5	9.6	7.3
Crude fibre	20.8	21.0	27.9	27.9	18.7	18.7	27.9	26.3

the nitrate content in the plant and the soluble carbohydrates. The carbohydrate content increased with increasing light intensity but the nitrate content, and to a lesser extent also the protein content, decreased. This was far more pronounced at a low nitrate concentration than at a high nitrate concentration. The fibre content appeared not to be affected at all.

The temperature also exerted its main influence on the nitrate and soluble-carbohydrate content but there was also a small but distinct influence on the protein and fibre content of the shoot. In the roots there was only an influence on the sugar content. The sugar content decreased with increasing temperature in all plant portions; the other components increased with increasing temperature.

The nitrate concentration had a very distinct influence on the nitrate content in all plant portions. This increased two to three times when the concentration in the solution increased fourfold. The carbohydrate content and the protein content were far less affected: the protein content increased with increasing nitrate concentration and the carbohydrate content decreased, but only at high light intensity. The crude-fibre content was not affected.

This preliminary experiment demonstrates that the external factors examined have different influences and the effect of one factor is influenced by the other two. At a high light intensity the nitrate concentration and the temperature influenced only the chemical composition and not the dry-matter production, although there were differences in dry-matter distribution and rate of tillering. That both factors did not run parallel in their influence may appear from the following comparison: –

	dm prod.	till.	sh/ro	NO_3-N	tot. N	TSC	RC
high N compared with low N	0	+	+	+	+	—	0
low temp. compared with high temp.	0	+	+	—	—	+	—

At a low light intensity the temperature also influenced the dry-matter production and so did the nitrate concentration, although to a lesser extent.

To go somewhat further into this matter the three factors were studied over a greater range, both separately and in combination.

Experiment 2

Eight earthenware containers were planted with tillers and cultivated on tap water in a greenhouse until a sufficient amount of new roots had been formed. All containers were then brought into a growth room at 15° C and the tap water was replaced by a two-salt nutrient solution of the following composition: $MgSO_4$ 0.5 mmol and KH_2PO_4 0.25 mmol plus a Hoagland A.Z addition. Two stock solutions were prepared, one consisting of $KNO_3 + Ca(NO_3)_2$, the other of KCl and $CaCl_2$. Of these two solutions different amounts were added to the different containers, so that the potassium content was always the same, but the nitrate content and the chlorine content varied. In this way the following nitrate concentrations could be established: –

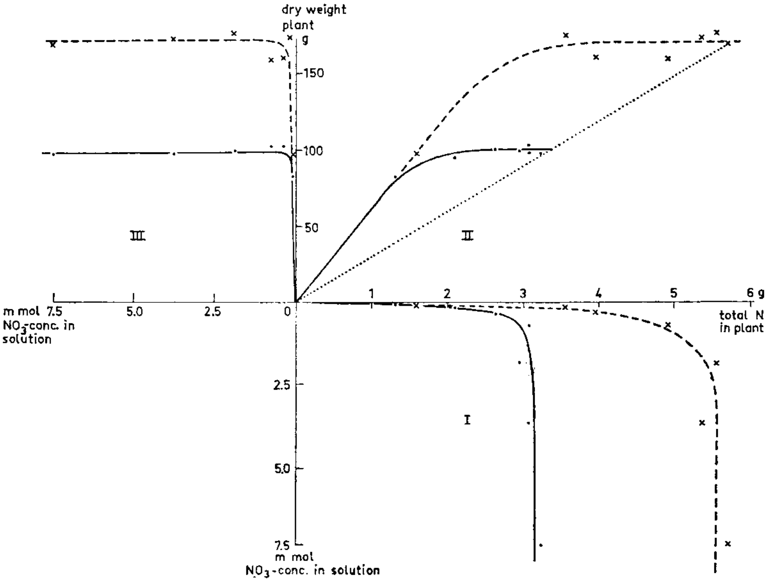
Container :	1	2	3	4	5	6	7	8
NO_3-conc.:	0.08	0.19	0.38	0.75	1.88	3.75	7.50	15.0 mmol

Four weeks after the start of the experiment half of the plants from each container were harvested and one week later the other half. The plants were divided into roots, stubble and leaves and the weight and the chemical composition of each portion was determined.

An exactly similar experiment was carried out some time later at a temperature of 25° C. The results of both experiments will be considered together.

The influence of the nitrate concentration of the culture solution on the amount of total nitrogen in the plant and on dry-matter production is given in FIG. 3. This

FIG. 3. The influence of the nitrate concentration in the nutrient solution on nitrogen uptake and dry-matter production



way of presenting the results is similar to that of FRANKENA and DE WIT (1958), who plotted the relation dosage-uptake-yield of several field experiments in this way.

The quadrant I shows the relationship between concentration and uptake for the two harvests at 15° C. In both cases a saturation curve was found, *viz.* the uptake increased with increasing nitrate concentration, but this increase gradually slowed down until above a certain value the concentration of the solution had no influence on the rate of uptake. It seems that the value of this saturation concentration increased slightly with the age of the plant. This kind of relationship between concentration and uptake has been found by many other workers in this field and is quite well known.

The quadrant III shows the relationship between the nitrate concentration in the solution and the dry-matter production for both harvests. Here a similar sort of curve was found, but only at the lowest nitrate concentration (0.1 mmol) was dry-matter production affected. Consequently there seems to be a nitrate concentration range in which the chemical composition is influenced, but production is not. This range lies approximately between 0.2 and 1.0—1.5 mmol nitrate in the solution. This explains why no influence on dry-matter production was found in experiment 1, since the concentrations used in this instance were 0.98 and 3.75 mmol nitrate.

The quadrant II shows the nitrogen content in the plant. It seems that there are two important threshold values. Below a concentration of 1.6 % nitrogen no growth seems to be possible and a value of 3.4 % appears to represent the maximum concentration which can be attained in the plant. The nitrate content can fluctuate between 2.4 and 3.4 % without influencing the rate of dry-matter production.

Similar trends were observed in the experiment conducted at 25° C, but the abovementioned thresholds were less easy to pinpoint since the data was rathermore variable.

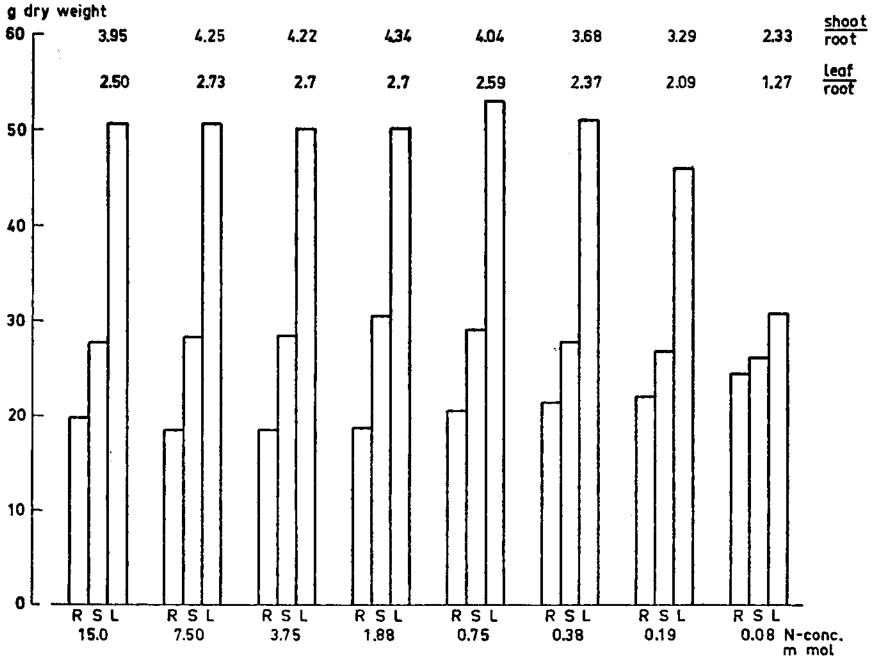
The influence of the nitrate concentration on the dry-matter distribution is given in FIG. 4 for the 15° C experiment. The calculated shoot/root ratios are given together with the actual weights of roots, stubble and leaves. From these data it is clear that the fall in shoot/root ratio with decreasing nitrogen concentration started below a concentration of 0.75 mmol and was caused by an absolute increase in root weight and below a concentration of 0.38 mmol N also by a decrease in leaf weight. There was no clear evidence to show that the stubble weight was affected by the nitrate concentration.

FIG. 5 shows the relationship between the nitrate concentration in the solution and the organic- and inorganic-nitrogen content in the plant. The organic-nitrogen figures have been obtained by subtracting the nitrate-nitrogen values from total nitrogen. The figure clearly shows that with decreasing nitrate concentration in the nutrient solution only the nitrate content in the plant diminished at first. The organic-nitrogen content remained the same until a concentration of 0.75 mmol nitrate in the solution was reached; below this value the organic-nitrogen content diminished too. It is also below this ion concentration that the dry-matter production was seriously affected.

The nitrate content in the stubble and the leaves increased with increasing nitrate concentration very rapidly at low concentration but at a constant rate above a concentration of 0.2 to 0.3 mmol up to the highest concentration used. The nitrate content in the roots tended to remain constant or only to increase slightly at concentrations above 0.4 mmol.

Since the inorganic nitrogen was only a minor part of the total nitrogen, this increase is not visible in FIG. 3 where the total amount of nitrogen is given for the whole plant.

FIG. 4. The influence of nitrogen concentration on the distribution of dry matter between roots (R), stubble (S) and leaves (L)



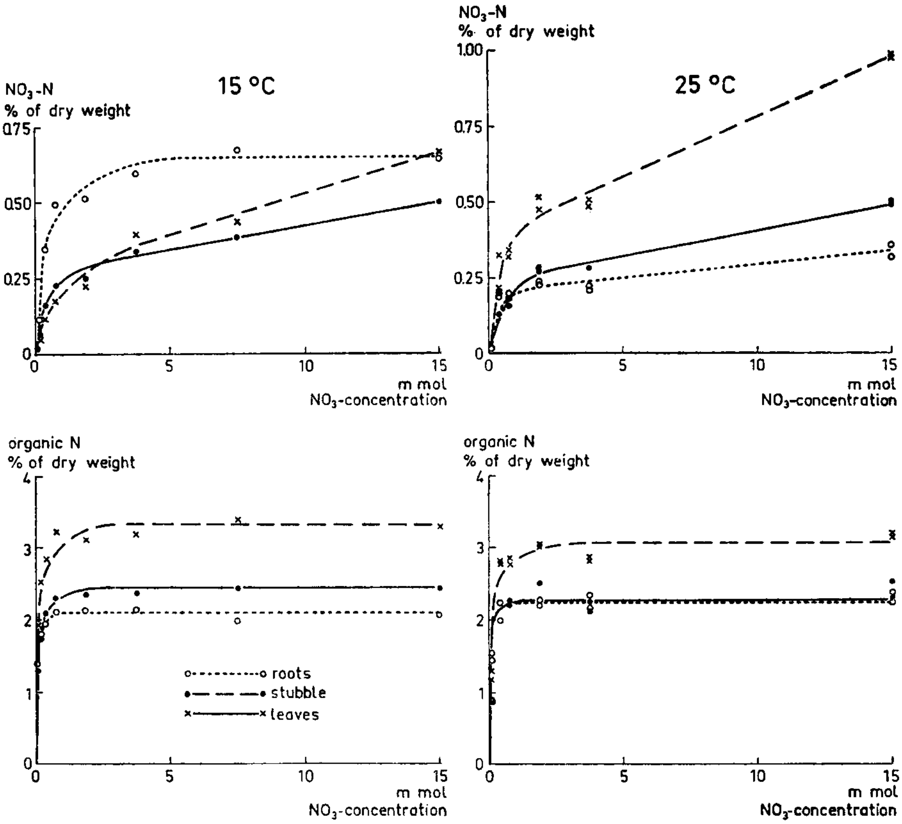
The differences between the two temperatures were only quantitative. There was no distinct difference in organic nitrogen, but the nitrate content in the roots at 15° C was much higher than at 25° C, so that at 15° C the highest value was found in the roots, except at very high and very low concentrations, whereas at 25° C the highest value was found in the leaves at all concentrations.

The relationship between the nitrate content in the plant and the soluble-carbohydrate content is shown in FIG. 6. At both temperatures the nitrate content could vary between wide limits before the carbohydrate content was affected, the limit being reached when the level of nitrate nitrogen fell below 0.3 %. The rate of dry-matter production was not influenced until the nitrate level fell below 0.1 % of the total dry matter. Between these two values chemical composition is influenced, but dry-matter production is not. Although the kind of relationship between the nitrate and carbohydrate content was the same for both temperatures, the two curves lie distinctly apart. Thus at the same nitrate content the sugar content was always higher at the lower temperature. It also seems that at a higher temperature higher nitrate concentrations can be reached in the plant.

The results of this experiment confirm and extend those of experiment 1, showing that the dry-matter production is only affected at a low nitrate concentration. Probably this value would have been even lower if a constant external concentration had been maintained by means of a flowing solution.

The observations on the effects of the different nitrate concentrations on the chemical composition at high light intensity were also confirmed with the exception that in this experiment temperature was found to have a greater effect on the nitrate

FIG. 5. The nitrate-nitrogen content and the organic-nitrogen content in relation to the nitrate concentration of the solution.
Left: temperature 15° C; right: temperature 25° C



content of the roots and a smaller effect on that of the shoots as compared to experiment 1. In the following experiments it will, however, be shown that as in experiment 1 the effect of external factors on the chemical composition of the roots was always small: possibly the differences observed in this experiment are related to the fact that nitrate was replaced by chloride only rather than by both sulphate and chloride as in the other experiments.

The influence of nitrogen concentration on chemical composition will be examined in more detail in the next experiment.

Experiment 3

Five earthenware containers were planted with tillers, as described in the foregoing experiment. They all remained in a greenhouse at 20° C until sufficient new material had been formed, when the plants were removed to a growth room at 20° C and grown on tap water. Two days later the five containers were distributed over the temperatures 10, 15, 20, 25 and 30° C and the tap water was replaced by the

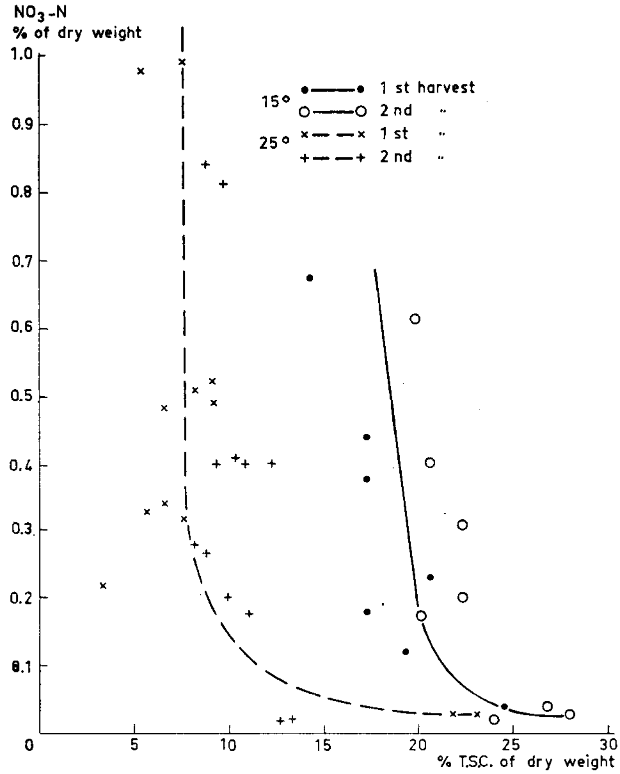


FIG. 6
The relationship between nitrate content and total soluble-carbohydrate content in the leaves

nutrient solution. By making the plants low in nitrogen before putting them at the different temperatures it was supposed that the plants on each container might attain the nitrogen status appropriate to the temperature in which they were growing. The nutrient solution of the containers was changed daily and care was taken that the new solution was brought to the right temperature beforehand.

After five days the normal solution was replaced by a nutrient solution without nitrogen in which KNO_3 and $Ca(NO_3)_2$ were replaced by equivalent concentrations of K_2SO_4 and $CaCl_2$. At this stage and at regular intervals afterwards plants were harvested.

For some unknown reason the plants on the containers differed considerably in size so that the data on dry-matter production were far more irregular than usual. The influence of temperature on dry-matter production is therefore better discussed in connection with the next experiment, as the results of the two experiments did not appear to be at variance with each other.

The data on chemical composition were fairly regular, as can be seen from FIG. 7, in which the nitrate content of roots, stubble and leaves is plotted against time. At the beginning of the N-free period the temperature effect on the nitrate concentration in the plant was very marked, especially in the leaf, where the highest concentration was around 0.7 % N and the lowest around 0.2 %. In the roots, however, the differences lay between 0.65 and 0.48 %. In all three plant fractions the highest

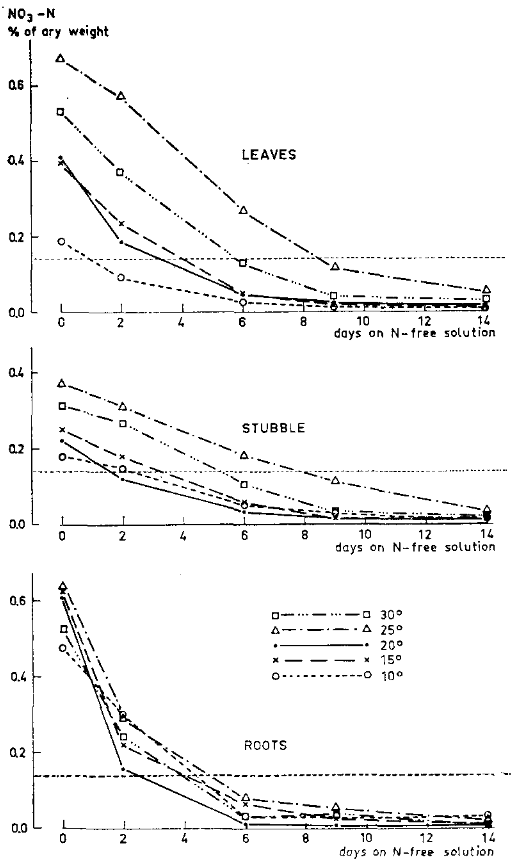


FIG. 7
The decrease in nitrate content with time on a N-free culture solution

concentration was obtained at 25° C and the lowest at 10° C, but the sequence of temperatures was not always the same in the different plant portions at the different harvests, the status of the roots being particularly liable to variation. The highest concentration remained at 25° C throughout the experiment, but after two days the lowest concentrations in the stubble and in the roots were recorded at 20° C. After two weeks on a nitrogen-free solution all plant portions had reached a nitrate-nitrogen content of below 0.05 %. In the leaves however, there was a great difference between the different temperature regimes in the time necessary to reach a certain concentration, *e.g.* 0.14 % (see discussion).

For the 25° C plants this was reached 8 days after changing the solution, while for the 10° C plants this process took only one day.

FIG. 8 shows the relationship between the nitrate and the soluble-sugar content in the plant. At an optimal nitrogen nutrition a distinct inverse relationship existed between both constituents in the leaves; in the stubble the same relationship existed, but within a much smaller range. In the roots the relationship was far less distinct, the nitrate content being high and the sugar content being low at all temperatures. When the nitrogen supply stopped, the relationship between nitrate and sugar content showed the same trend in all cases. At first the nitrate concentration fell without

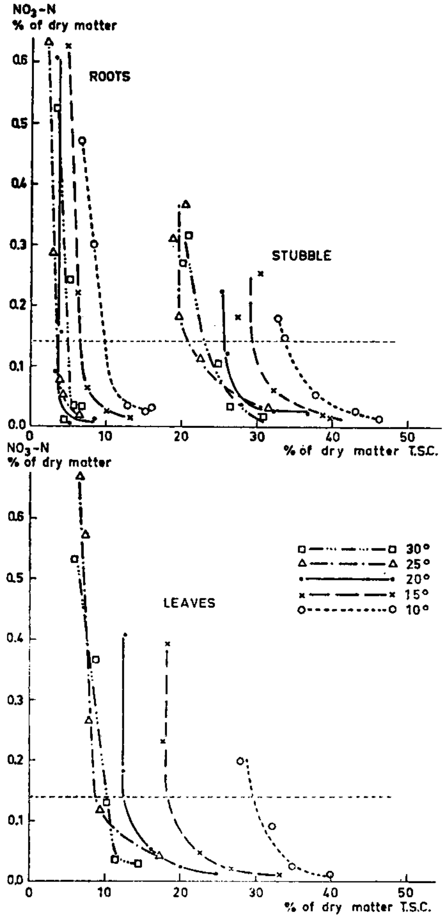
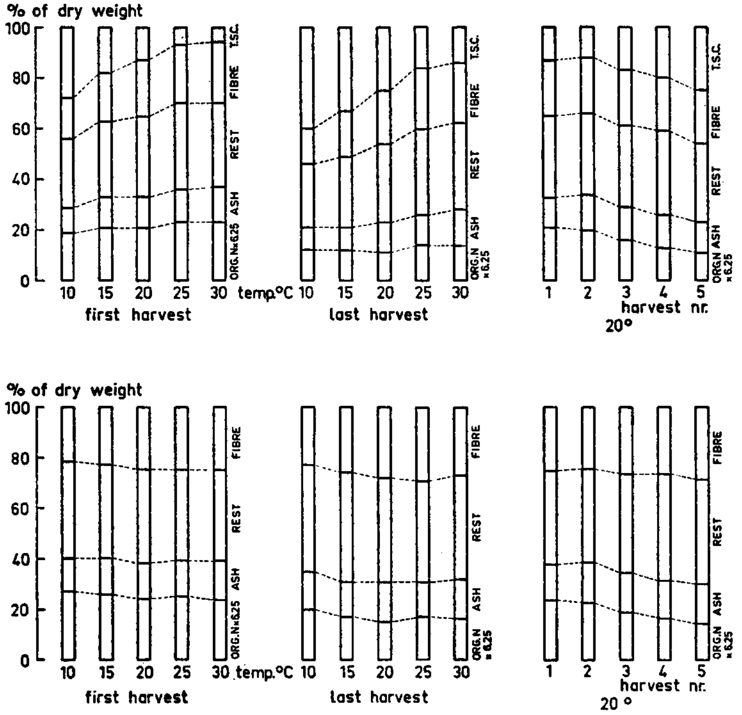


FIG. 8
The relationship between nitrate content and total soluble-carbohydrate content in roots, stubble and leaves during nitrogen starvation

any concomitant change in the sugar content. Below a content of 0.02 % nitrate nitrogen in stubble and leaves and 0.1 % in the roots, the sugar content increased with decreasing nitrate content. This took place slowly at first but became more rapid at low nitrate concentrations when values of 46 % in the stubble and 40 % in the leaves (10° C) were reached. Notwithstanding this the differences in nitrate concentration caused by the different temperatures disappeared gradually, while the differences in sugar content remained throughout the experiment.

The influence of the temperature on the main plant constituents is given in FIG. 9. Crude-fibre and ash content were determined by the usual method. Instead of multiplying the total-nitrogen content by 6.25 to obtain the crude-protein value, the organic-nitrogen content has been multiplied by 6.25 to get a value which is indicated as "org. N × 6.25". "Rest" designates the plant constituents not determined, consisting for an important part of hemicelluloses and pentosans. The left-hand series of histograms in the upper row gives the constituents at the different temperatures at the beginning of the experiment. The sugar content decreased very conspicuously with

FIG. 9. The influence of temperature and nitrogen nutrition on the main leaf constituents (see text)
Upper half: calculated on normal dry weight
Lower half: calculated on sugar-free dry weight



increasing temperatures and the other constituents increased, the greatest change being found with the fibre content. Since the greater part of the soluble carbohydrates can be considered as reserves, one may ask how great the temperature influence is on the sugar-free plant. Accordingly the plant constituents are given in the left-hand series of histograms of the lower row as a percentage of the sugar-free dry weight. It now becomes clear that the influence of the temperature on chemical composition is negligible, none of the constituents changing more than 5 % in value. Thus the temperature only had a direct effect on the sugar content; the influence on the other constituents was indirect. The same situation arises at the end of the experiment under conditions of nitrogen shortage (two middle histograms). In comparing the first and the last harvest, it can, however, be seen that the content of organic N decreased during the experiment. The changes in plant constituents during the experiment at a temperature of 20° C are accordingly given in the right-hand histograms again calculated both on total dry weight and on a sugar-free basis.

It can now be seen that the shortage of nitrate not only affected the sugar content directly but also the content of organic nitrogen and undetermined substances leaving the crude-fibre and ash content unchanged. That the shortage in nitrate supply not only caused an increase in sugar content but also a decrease in organic nitrogen and a decrease in some unknown constituents follows also from TABLE 2 in which the constituents calculated on the sugar-free dry weight are averaged over the different temperatures at the beginning and the end of the experiment.

These results are in complete agreement with those of the foregoing experiment. Whether the changes in nitrate content of the plant are brought about by cultivating them on nutrient solutions of different concentrations during prolonged periods or by analysing them at regular intervals during a relatively short period without nitrate supply, the changes in chemical composition are the same. It is only at high temperatures that the relationship between the nitrogen and sugar content is not as one would expect. In a repetition of this experiment the same results were found; usually the lowest sugar content was not found at the highest temperature but at 25° C and in one case even at 20° C. This was mostly, but not in all cases, inversely correlated with the nitrate content. Apparently the sugar content is not directly and exclusively influenced by the temperature in such a way that it always decreases as the temperature increases.

To gain more insight into this matter the influence of the temperature on chemical composition was studied at two different light intensities.

Experiment 4

Tillers were planted on one-litre pots and cultivated in the greenhouse until suffient new roots were formed. The plants were then brought into a growth chamber at 20° C, the nutrient solution was replaced by tap water and half of the pots were shaded to 2/5 of the normal light intensity.

TABLE 2. The main leaf constituents calculated as a percentage of the sugar-free dry weight

	Org. N × 6.25	Ash	Rest	Fibre
First harvest	25.2	14.0	36.5	24.3
Last harvest	17.1	14.8	41.4	26.7

Two days later a number of plants from each light intensity was harvested and the other plants were distributed between five rooms with temperatures of 10, 15, 20, 25 and 30° C. At the same time the tap water was replaced by the normal nutrient solution. From each of the ten groups plants were harvested at regular intervals, weighed and analysed.

At the time of the first harvest, four days after the start of the temperature treatments, there were already very distinct differences in chemical composition, which did not change much thereafter. The dry weight and the number of tillers increased fairly regularly, so that the results from the harvest at the end of the experiment may be considered representative of the dry-matter production and the chemical composition under the different treatments (TABLE 3).

At the highest light intensity the optimum temperature for dry-matter production was 25° C; at the lower light intensity it was 20° C. This shift in the optimum temperature towards higher values with increasing light intensity has been found repeatedly, and occurs also in other plant species. Moreover the differences in dry weight due to the different temperatures were much greater at high light intensity than at low light intensity. The shoot/root ratio figures are rather irregular. There is a slight tendency for this ratio to decrease with increasing temperature and with increasing light intensity, but the results do not allow any definite conclusions to be drawn.

TABLE 3. The influence of temperature on production and distribution of dry matter (upper part) and on chemical composition (lower part) at two different light intensities

Light intensity:	2×10^4 ergs cm^{-2} sec^{-1}					5×10^4 ergs cm^{-2} sec^{-1}				
Temperature (°C):	10	15	20	25	30	10	15	20	25	30
Dry weight (g)										
Roots	3.11	2.50	3.92	3.44	3.39	3.48	7.07	7.42	11.19	6.73
Stubble	6.31	5.50	4.79	2.92	2.37	6.85	11.57	10.57	11.73	7.98
Leaves	11.70	15.96	15.99	13.41	12.11	13.62	23.09	29.39	32.83	19.61
total:	21.12	23.96	24.70	19.77	17.87	23.95	41.73	47.38	55.75	34.32
Shoot/root ratio	5.79	8.58	5.30	4.75	4.27	5.88	4.90	5.39	3.98	4.10
Shoot/root ratio (preceding harvest)	5.92	5.83	5.59	5.12	5.08	6.17	4.89	5.06	4.35	5.56
Roots										
NO_3-N	0.65	0.76	0.72	0.73	0.51	0.52	0.54	0.64	0.48	0.53
Total N	3.18	3.20	3.09	2.93	2.82	3.26	3.18	3.23	3.06	2.96
T.S.C.	13.5	9.9	8.9	6.4	7.1	13.8	10.0	9.1	7.5	9.8
Crude fibre	—	—	24.0	—	—	—	24.3	24.6	24.5	22.5
Stubble										
NO_3-N	0.22	0.37	0.47	0.53	0.46	0.17	0.25	0.27	0.23	0.28
Total N	2.50	2.72	2.74	2.86	3.12	3.02	2.90	2.64	2.67	2.78
T.S.C.	40.8	30.9	23.2	16.1	16.2	39.8	30.9	27.5	24.2	25.3
Crude fibre	14.0	17.1	20.6	—	—	13.0	16.5	18.5	21.0	19.0
Leaves										
NO_3-N	0.17	0.50	0.57	0.70	0.71	0.13	0.25	0.31	0.27	0.38
Total N	3.30	3.74	3.82	4.03	3.82	2.98	3.60	3.63	4.03	3.97
Crude fibre	24.6	13.9	9.4	5.7	4.5	33.4	20.3	15.5	10.8	7.4
T.S.C.	17.3	21.3	23.2	24.0	24.4	14.7	17.7	19.5	21.2	22.0

The data of the preceding harvest, which is also given in the table, do however show the same trend.

The nitrate concentration in the leaves increased with temperature, especially at lower values and at low light intensity. This was also the case in the stubble, although the range was smaller, but in the roots temperature was not found to have an influence on the nitrate concentration.

A distinct influence of temperature and light intensity on the soluble-carbohydrate concentration was also observed. The sugar content decreased at both light intensities with increasing temperature up to 25° C in roots and stubble and to 30° C in the leaves. It was always higher at the higher light intensity, the differences being greatest in the leaves.

The relationship between the nitrate content and the soluble-carbohydrate content is given in FIG. 10 for the three plant portions and for all harvests. The data from the first harvest of experiment 3 is also included. The data at low light intensity is indicated by crosses, that at high light intensity by dots. The relationship remains the same whether, for example, a low carbohydrate content is reached by a low light intensity or a high temperature. No evidence of such a relationship was found in the roots. The data of experiment 3 fit very well with those of experiment 4 as far as the leaves are concerned; for the stubble and the roots

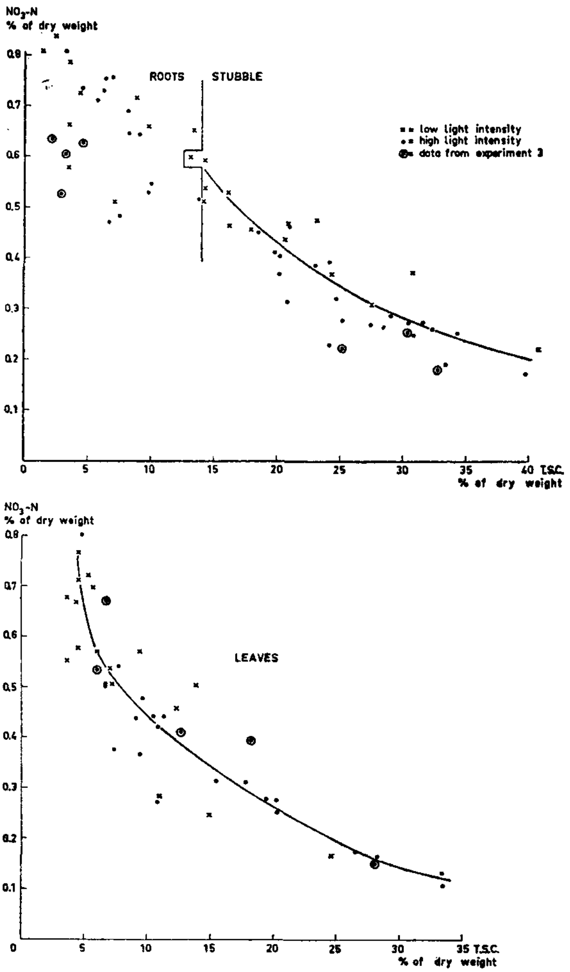


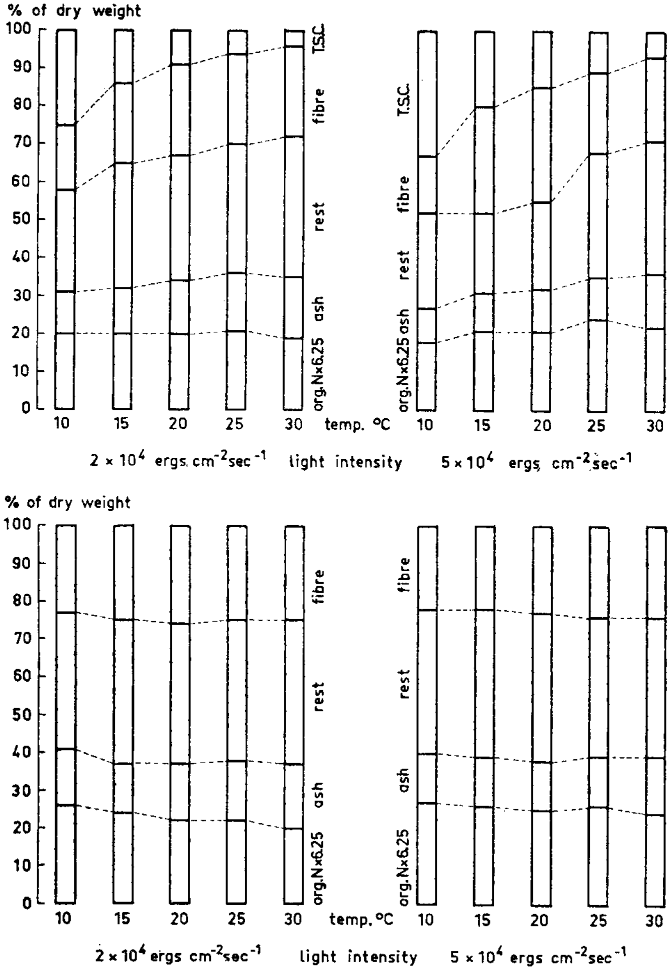
FIG. 10
The relationship between nitrate content and total soluble-carbohydrate content in roots, stubble and leaves for different temperatures and light intensities

they fall below the average values. Data from other experiments also fit reasonably well with the data given in the figure.

The upper part of FIG. 11 also shows the percentage contribution of the other leaf constituents to the total dry weight. That the changes in these constituents are caused only by changes in the sugar content, may be seen by calculating their relative contributions on a sugar-free dry weight (lower part of diagram). They are thus independent of temperature or light intensity.

Thus this light-intensity experiment has demonstrated that for a given concentration of nitrate in the nutrient solution a relationship exists in the stubble and leaves between the nitrate concentration and the soluble-carbohydrate content, and that this relationship is independent of external conditions. The results of the foregoing experiments are therefore fully confirmed. Once again the sugar content reached a minimum at 25° C in the roots and stubble; for the leaves this minimum was reached at 30° C.

FIG. 11. The influence of temperature and light intensity on the main leaf constituents
Upper half: calculated on normal dry weight
Lower half: calculated on sugar-free dry weight



The next experiment shows what happens when nitrogen is withheld from plants at a series of light intensities and in complete darkness.

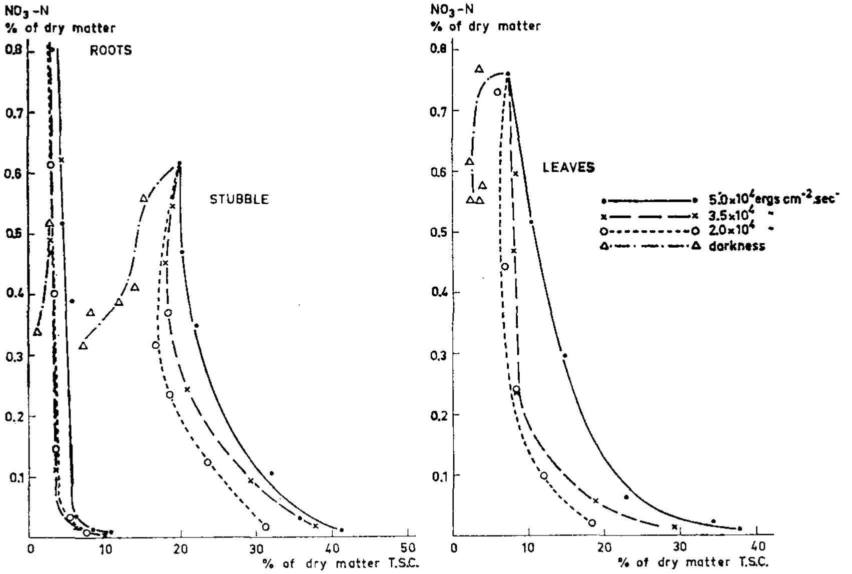
Experiment 5

Plants were grown in a growth room at 20° C and full light intensity until they were ready for the experiment. They were then divided into four groups receiving the following light intensities: 5.0, 3.5 and 2.0 × 10^4 ergs cm^{-2} sec^{-1} and darkness, the temperature remaining at 20° C for all groups. At the same time the normal nutrient solution was replaced by one without nitrogen in the way described in experiment 3. Plants were taken for analysis at the start of the experiment and 1, 3, 6, 10 and 15 days thereafter.

The increase in dry weight during this period for the four light intensities was 42.29, 28.03, 14.29 and —6.61 g respectively for high and low values. If one assumes that all plants have lost 6.61 g as respiration the true photosynthesis during this period was 48.90, 34.64, 20.90 and zero g respectively, or in the ratio of 5.0 : 3.5 : 2.1 : 0.0, which is almost exactly the ratio of the light intensities used.

The relationship between the sugar content and the nitrate content is given in FIG. 12.

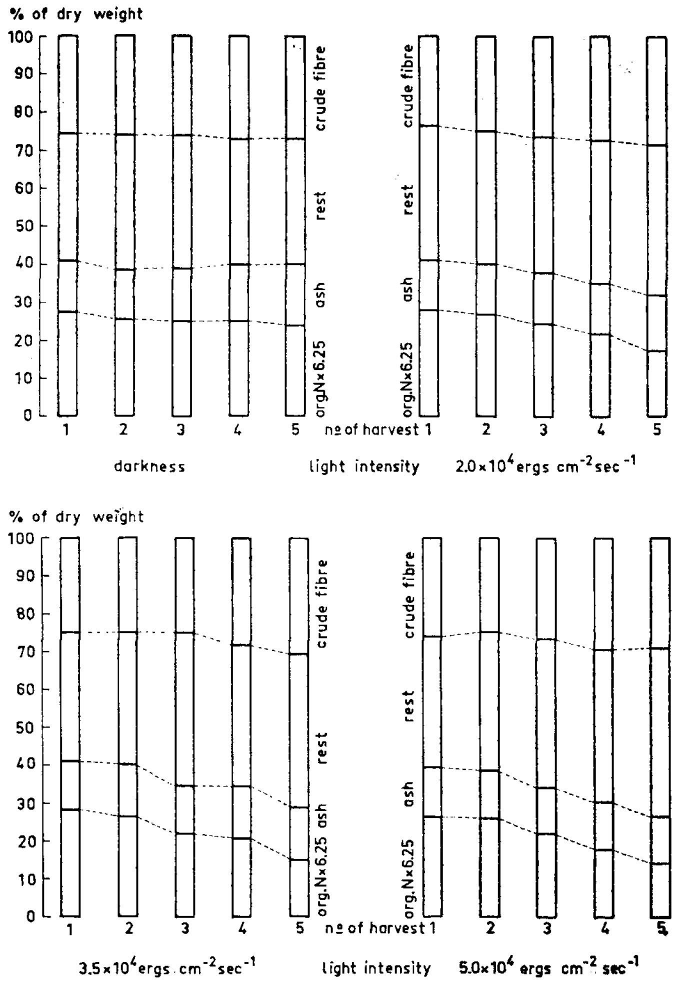
FIG. 12. The relationship between nitrate and total soluble-carbohydrate content in roots, stubble and leaves during nitrogen starvation at different light intensities



The higher the rate of dry-matter production, the more rapidly was the nitrate used and the more rapid was the increase in soluble-sugar content. The sugar concentration reached at the end of the experiment was highest at the highest light intensity; nitrate was virtually absent, except in darkness. In the stubble in particular the sugar concentration seemed to decrease slightly at the beginning of the low light intensity treatment. It is thought that when the plants were brought from the normal light intensity of 5.0 × 10^4 ergs cm^{-2} sec^{-1} to a lower value the rate of photosynthesis diminished, whereas the conversion of the reserves into structural components might go on for a while at the normal rate causing the sugar content to drop. The lower the light intensity, the more marked was this initial drop. This was followed by a subsequent increase in concentration due to nitrogen shortage except in the dark, where it remained at a rather low value, especially in the leaves and the roots. Together with these changes in soluble sugars went an initial nitrate reduction after which the nitrate concentration remained at a rather high level in all plant portions.

FIG. 13 gives the chemical composition of the leaves calculated on a sugar-free dry weight basis for the four light intensities and the five harvests. At the normal light intensity of 5 × 10^4 ergs cm^{-2} sec^{-1} the trend is exactly the same as in FIG. 9 for

FIG. 13. The influence of light intensity on the main leaf constituents, calculated on sugar-free dry weight



the successive harvests. The organic-nitrogen content decreased during nitrogen starvation and in general the "rest" increased. With decreasing light intensity these changes became smaller until no change in chemical composition could be observed in darkness.

4. Discussion

4.1. The influence of the nitrate concentration

From experiment 2 it appears that the nitrate concentration in the nutrient solution only affects the rate of dry-matter production when it is very low. At concentrations of 0.2 mmol or higher the dry-matter production is not influenced. The same shape of curve was found by HYLTON *et al.* (1964) for the relation between dry weight and nitrate concentration in the plant. The value of 0.2 mmol would have been still lower if a streaming solution of constant concentration had been used. However, FIG. 4 shows that the concentration nonetheless influences the distribution of dry matter up to a concentration of 0.75 mmol nitrogen. This is somewhat unexpected, as one may assume that the rate of dry-matter production would be influenced when a greater part of the total amount of dry matter produced goes to non-photosynthesizing tissue.

The rate of uptake of nitrogen increases with increasing nitrate concentration in the solution; rapidly at first, but then at a steadily decreasing rate until an external concentration is reached above which the uptake is not influenced. The curve representing this relationship resembles those found by other workers (VAN DEN HONERT and HOOIMANS, 1955; LYCKLAMA, 1963). The concentration at which the maximum uptake was reached increased somewhat with the age of the plant, rising from ca. 1.5 mmol at the first harvest to ca. 3.5 mmol at the second harvest. Possibly this shift is caused by the fact that the older plant absorbs the nitrate at a faster rate, so that with such a plant the mean concentration of the solution during an uptake period is lower than with a younger plant, when starting with the same concentration of the fresh solution. LYCKLAMA (1963) working with seedlings found a saturation value of 0.2 mmol.

In studying the relationship between concentration and uptake it is usually found that the uptake does not increase above a certain value. When the amount of nitrate in the plant was analysed, however, it appeared that the internal concentration increased with increasing outside concentration up to the highest value used. An attempt was made to increase the external concentration still further, but this appeared to diminish the growth rate, possibly because the osmotic value of the solution had become too high. The data from FIG. 5 suggests that after a rapid increase of both the organic and nitrate nitrogen the former becomes constant at a certain external concentration, but that the nitrate concentration in the above-ground parts of the plant increases linearly with increasing external concentration.

Since the inorganic nitrogen is only a small fraction of the total nitrogen taken up, this increase in nitrate concentration in the plant is scarcely evident in FIG. 3. LYCKLAMA (1963) did not analyse the plant but the data presented in his figure 9 also suggests a small but steady increase of the nitrate uptake with increasing concentration.

Apparently this increase in nitrate concentration in the plant has no further influence on the other plant constituents. It forms a kind of reserve nitrate which is rapidly depleted when nitrate is withheld from the external solution (FIG. 7).

The speed of this depletion depends on the rate of growth but is apparently also promoted by low temperature, since the temperature at which the nitrate reserve is most rapidly exhausted lies at 10° C for the stubble and at 20° C for leaves and roots, followed by 10° C. Although the light intensity might influence nitrate reduction by itself (KESSLER, 1964), this influence cannot be distinguished from that associated with rate of growth in the present experiment. With all light intensities used the nitrate concentration reaches the same very low value in the end, except in darkness where the depletion comes to a standstill at a fairly high nitrate level. The absence of nitrate reduction in the dark had been found repeatedly and is usually ascribed to lack of carbohydrates. It has been demonstrated in cutting experiments at different reserve levels that the soluble-carbohydrate level never becomes zero (DEL POZO IBAñEZ, 1963) and it was concluded that the remaining sugars are essential for the functioning of the cell. In the same way the carbohydrate level at which nitrate reduction stops at darkness may also be the minimum level for normal plant life.

Until the nitrate concentration falls to 0.2 % of the dry weight it has little influence on the sugar content (FIG. 6, 8 en 12). Below this value, however, the sugar content rises rapidly with decreasing nitrate content. As already stated the rate of dry-matter production seems only to be affected by a still lower concentration. Thus there seems to be a concentration range in which the chemical composition is affected, but the dry-matter production is not (see FIG. 1 and TABLE 1).

It is however not only the soluble-carbohydrate concentration that is influenced by nitrate nutrition, for when the other organic constituents are calculated on a sugar-free dry weight basis, differences remain in the crude-protein content and in the fraction which was not analysed, designated as "rest" (FIG. 9). In TABLE 4 the tiller weights from experiment 3 and 5 are compared with those from experiment 4 in which the nitrogen nutrition was continuous throughout the experiment.

TABLE 4. Dry weight per tiller with and without nitrate in the nutrient solution

Days after start of experiment	With nitrate in solution, experiment 4 (mg)	Without nitrate in nutrient solution				
		experiment 3 (mg)	experiment 5			
			5.0 ergs (mg)	3.5 ergs (mg)	2.0 ergs (mg)	dark (mg)
0	87	113	81	87	77	73
2	100	122	99	84	85	74
6	99	127	117	98	96	78
9	106	146	156	134	109	73
14	114	148	178	167	144	83

From these data it follows that the rate of tiller formation is diminished when nitrogen is withheld from the solution. This would mean the formation of fewer growing points and relatively less tissue extremely rich in protein. The same can be observed in experiment 1 (FIG. 1), in which the nitrate concentration had no influence on the rate of dry-matter production but a distinct effect on tiller formation. It must be stated, however, that no differences in tiller weight were found in experiment 2 in which the plants had grown on solutions with different nitrogen concentrations from the beginning and in which, nevertheless, the same differences in chemical composition occurred. This experiment differed also from the others in that the plants were cultivated on earthenware containers instead of pots. In this way a kind of closed sward is formed, which might change the chemical composition as compared with plants growing more freely (ALBERDA, 1965). In addition, nitrate was replaced by chloride only, whereas in other experiments it was replaced by both sulphate and chloride.

4.2. The influence of temperature and light intensity

Both these factors have an influence on dry-matter production and chemical composition. They interact in determining dry-matter production so that the optimum temperature is dependent on the light intensity used and the effect of the light intensity is different at different temperatures (TABLE 3). This can be explained by the fact that the light intensity influences only the rate of photosynthesis and not the respiration and that the temperature influences both in a different way. The effect of these influences can be seen in a model plant, for which the relationship between photosynthesis and light intensity is shown schematically in FIG. 14 for the tempera-

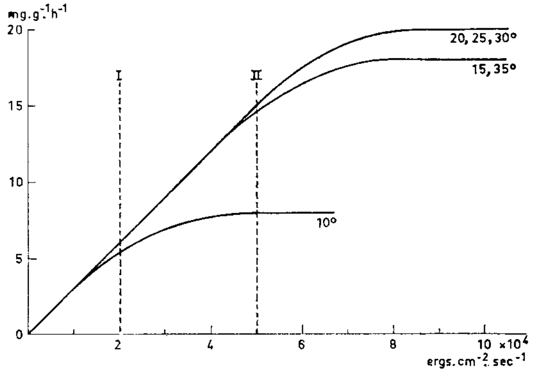


FIG. 14
Diagrammatic relationship between photosynthesis and light intensity at different temperatures

tures used in the experiments, and assuming that the other items are as follows: – Total weight 20 g; weight of assimilating tissue 12 g; rate of respiration at 25° C 1 mg. g^{-1} . h^{-1} and Q_{10} of the respiration 2.0.

For a light period of 18 hours the values for true photosynthesis, respiration and net photosynthesis are given in TABLE 5 for the two light intensities indicated in the figure.

Just as in experiment 4 the temperature optimum shifts to a lower value at lower light intensities, apparently caused by the fact that the respiration, which strongly increases with increasing temperature, becomes of relatively more importance at lower light intensities.

The same was found by MITCHELL and LUCANUS (1962) for different day-lengths for both dry-matter production and rate of tillering. The fact that the temperature had no effect on dry-matter production in experiment 1 (FIG. 1a) was apparently caused by the fact that the two temperatures used (15° and 25° C) were on both sides of the optimum.

TABLE 5. Photosynthesis and respiration at different temperatures calculated for a model plant (see text) for 2 light intensities. The ratio of apparent photosynthesis at the two light intensities is compared with the same ratios from experiments 1 and 4 (two harvests)

Temperature (°C):	10	15	20	25	30	35
Light intensity II						
True photosynthesis (mg)	1728	3132	3240	3240	3240	3132
Respiration (mg)	156	240	336	480	672	960
Apparent photosynthesis (mg)	1572	2892	2904	2760	2568	2172
Light intensity I						
True photosynthesis (mg)	1188	1296	1296	1296	1296	1296
Respiration (mg)	156	240	336	480	672	960
Apparent photosynthesis (mg)	1032	1056	960	816	624	336
Light intensity II/I	1.52	2.74	3.03	3.38	4.12	6.46
Experiment 1	—	2.34	—	4.82	—	—
2 last harvests experiment 4	1.62	3.79	5.46	3.12	7.62	—
	1.30	2.47	2.77	5.42	3.74	—

In the example given the ratio of the light intensities was 2.5 just as in experiment 4. The highest light intensity used was 5.10^4 ergs cm^{-2} sec^{-1} which lies distinctly below the saturation point at temperatures around 20° C (GAASTRA, 1959). Hence it can be supposed that the true photosynthesis increases linearly with the light intensity in the range used. At a temperature of 20° C the ratio between the actual dry-matter production at the two light intensities will be greater than 2.5, since photosynthesis has had to be reduced in both cases by the same value for respiration. On account of the increasing respiration this ratio between the dry-matter production at the two light intensities will increase with increasing temperature, as can be seen from the example given in TABLE 5. The ratios calculated from experiment 1 (15 and 20° C) and experiment 4 (last and last but one harvest) are also given. Although the figures are irregular at 25° and 30° C, the trend is the same: the ratio increases with increasing temperature. At 10° C the calculated and the observed ratios were both less than 2.5. It can be concluded that in that case the photosynthesis at the highest light intensity is less than indicated by a linear relationship, in other words, the highest light intensity is already above the saturation point as is indicated in FIG. 14 and as was actually found by MILNER and HIESEY (1964).

The question of whether or not there is an influence of temperature and light intensity on the shoot/root ratio will not be discussed here as the data on this point (FIG. 2 and TABLE 2) are very irregular. The fact that the ratio tends to decrease with increasing temperature seems not to be in agreement with the results of many other workers in this field although similar trends have been observed by TROUGHTON (1961). Further work is in progress.

The effects of temperature and light on chemical composition are confined to the sugar content and the nitrate content of the plant. The other plant constituents remain the same under all conditions when calculated on a sugar-free dry weight.

This fits in with the fact that no distinct differences were found in tiller weight, indicating that a factor only influences the chemical composition of the organic non-sugar components if it influences tiller weight.

The possibility still exists that the effect of temperature on the nitrate content in the plant is indirect, the transpiration being higher at a high temperature than at a low temperature, resulting in a difference in the amount of nitrate carried into the leaves by the transpiration stream. Although the relative humidities at the different temperatures were such that the diffusion-pressure deficit between the air and a free water surface was approximately the same, an additional experiment was carried out at 30° C in which the nitrate concentration in plants at high relative humidity was compared with that in plants at low relative humidity. Since there were no differences between both groups it can be said that the differences in nitrate concentration in the upper plant parts were not caused by differences in transpiration. Another explanation of the difference in nitrate concentration will be referred to below.

With regard to the influence of the temperature on the carbohydrate content it seems that with values below the optimum for dry-matter production the production of carbohydrates drops less than the consumption of carbohydrates in growth and respiration, causing the reserves to rise with decreasing temperature. Above the optimum temperature the rate of dry-matter production falls but the respiration rises. In this range the effect of temperature on the soluble-sugar content is far less constant than at lower temperatures. Sometimes the lowest sugar content is found at the optimal temperatures, but more often the level decreases with increasing temperature up to the highest value used, the situation apparently depending on whether the increase in respiration prevails or the decrease in growth rate.

A higher light intensity gives a higher rate of sugar formation, but also a higher rate of dry-matter production, *i.e.* of consumption of sugars. Apparently the production rate is higher than that of consumption since the sugar content increases with increasing light intensity at all temperatures (TABLE 3).

Since the other plant constituents remain the same it might be supposed that with increasing reserve level the water content of the cell is being partly replaced by sugar. That it is not as simple as that appears from the fact that the lowest dry-matter content is always correlated with the highest growth rate but not always with the lowest sugar content. FIG. 15 A, B, C shows the percentage dry matter from three different experiments calculated from the normal fresh and dry weights and from the sugar-free weights, the latter procedure being based on the idea that the greater part of the soluble sugars are reserves and not essential for the functioning of the cell. The dry-matter content on a sugar-free basis is not the same for all temperatures, suggesting that the temperature has an influence on the water content of the cell by means of an influence on cell size. Attempts to get an impression of cell size at different temperatures by counting the number of stomata in the optical field of a microscope failed on account of the fact that the stomata are only found along the deep ridges on the upper side of the leaf so that it is very difficult to make them all clearly visible. Accordingly another method was adopted. Seen from the lower epidermis the parenchyma cells between the vascular bundle and the epidermis are clearly visible. For each temperature the number of cells along the diameter of the optical field was counted for the second vascular bundle from the edge of the leaf. Although there was a considerable variation in cell size the mean of all measurements indicated the smallest number of cells at the highest growth rate (FIG. 15 D).

This fits in with MITCHELL's data (1956). He found that both the dry-matter percentage and the dry weight per unit leaf area had the lowest value at optimal shoot growth.

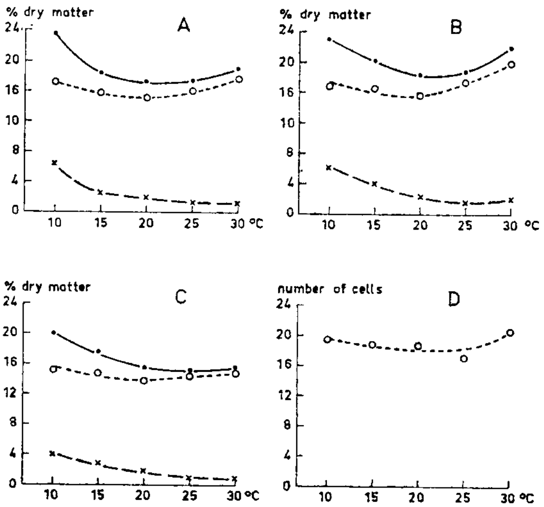


FIG. 15
The percentage dry matter calculated for normal dry weight and sugar-free dry weight, the difference being the sugar effect on the dry-matter content
A, B and C: results of three different experiments
D: the influence of temperature on cell size (expressed as number of cells per unit length

The relation between the sugar content and the nitrate content is very striking. When the data of a large number of experiments carried out at one external nitrate concentration, but at different temperatures and light intensities, are put together it appears that for one particular sugar concentration in the plant there is always a definite nitrate concentration (FIG. 10). Similar results were found by BATHURST and MITCHELL (1958) and by NOWAKOWSKI (1962). Apparently it is not the nitrate concentration that determines the sugar concentration as the nitrate value can easily be changed by means of the outside concentration without influencing the sugar content. Perhaps a better assumption would be that it is the other way round, although it may of course be possible that the level of both is determined by a third factor. When, as a consequence of a difference in temperature (see TABLE 1) the sugar concentration in the leaves of a plant I is higher than that of a plant II, it may be assumed that the sugar stream from the leaves downward is also greater in plant I than in plant II. If the nitrate uptake is the same for both plants and the nitrate ions are reduced and incorporated into organic compounds on their way up it can be supposed that at a certain height of the plant more nitrate will be reduced in plant I than in plant II. In this way it becomes possible to understand why differences in sugar content are always connected with differences in nitrate content and why these differences become smaller in the lower plant portions, especially in the roots.

Under field conditions the same relationship between nitrate concentration and sugar content can be observed as under controlled conditions (ALBERDA and SIBMA, 1962). A value of 100 milli-equivalents nitrate per kg dry matter is taken as a critical value (ALBERDA, 1965). This value is indicated in FIG. 7 and 8 as a dotted line. Above this value both the dry-matter production and the chemical composition will scarcely be influenced. It has been shown under field conditions that the sugar concentration was then slightly below 8 % of the dry matter in the leaves but that short periods with a nitrate content below the critical value coincided with peaks in the carbohydrate content. In contrast, however, to he results obtained in growth chambers the other chemical constituents will change at optimal nitrogen nutrition in the field on account of ageing, which will cause the fibre content to rise and the organic-nitrogen content to drop (ALBERDA and SIBMA, 1962). The reason for this difference is that the plant grows mainly by the formation of new tillers when it can grow freely, as in the growth chambers, but that it grows by an increase in length of the existing tillers under sward conditions.

Since a protein content of 15 % of the dry matter would be sufficient for the grazing animal a higher sugar content that the 8 % found at optimal nitrate nutrition would be most welcome, both from the point of feeding value (less losses by the animal in the form of urea) and for silage making.

Although the experiments have shown that the sugar concentration can rise considerably without influencing dry-matter production, the necessary control of the nitrate concentration seems to be impossible in practice.

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REFERENCES

ALBERDA, TH. 1957 The effects of cutting, light intensity and night temperature on growth and soluble carbohydrate content of *Lolium perenne* L. *Plant and Soil. 3,* 199—230.

—— 1958 The phytotron of the I.B.S. at Wageningen. *Acta bot. neerl.* No. 7, 265—277.

—— 1960 The effect of nitrate nutrition on carbohydrate content in *Lolium perenne. Proc. 8th internat. Grassl. Congr.*, 612—615.

—— 1965 The problems of relating greenhouse and controlled environmental work to sward conditions. *J. Brit. Grassl. Soc. 20,* 41—48.

—— and L. SIBMA 1962 Dry matter production and light interception of crop surfaces. II. Relation between rate of growth and length of grass. *Jaarb. I.B.S. Wageningen, 1962.* 47—58.

BATHURST, N. O., and K. J. MITCHELL 1958 The effect of light and temperature on the chemical composition of pasture plants. *N.Z.J. Agric. Res. 1,* 540—552.

DEL POZO IBAñEZ, M. 1963 The effect of cutting treatments on the dry matter production of *Lolium perenne* L. and *Dactylis glomerata* L. *Versl. Landbk. Onderz.* No. 69, 17. 74 pp.

DIJKSHOORN, W., en J. E. M. LAMPE 1960 Een nitraatbepaling voor kleine monsters gewas, grond of voedingsoplossing. *Jaarb. I.B.S. Wageningen, 1960.* 139—141.

DEIJS, W. B. 1961 De bepaling van totaal-stikstof in gewasmonsters inclusief nitraat-stikstof. *Jaarb. I.B.S. Wageningen, 1961.* 89—91.

FRANKENA, H. J., and C. T. DE WIT 1958 Stikstofbemesting, stikstofopname en grasgroei in het voorjaar. *Landbk. Tijdschr. 70,* 465—472.

GAASTRA, P. 1959 Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. *Meded. Landb. hogesch. Wageningen.* 59(13) 1—68.

HONERT, T. H. VAN DEN, and J. J. M. HOOIMANS 1955 On the absorption of nitrate by maize in water culture. *Acta bot. neerl. 4,* 376—384.

HYLTON, L. O., D. E. WILLIAMS, A. ULRICH and D. R. CORNELIUS 1964 Critical nitrate levels for growth of Italian ryegrass. *Crop Sci. 4,* 16—19.

KESSLER, E. 1964 Nitrate assimilation by plants. *Ann. Rev. Plant Physiol. 15,* 57—72.

LYCKLAMA, J. C. 1963 The absorption of ammonium and nitrate by perennial ryegrass. *Acta bot. neerl. 12,* 361—423.

MILNER, H. W., and W. M. HIESEY 1964 Photosynthesis in climatic races of Mimulus. I. Effect of light intensity and temperature on rate. *Plant Physiol. 39,* 208—213.

MITCHELL, K. J. 1956 Growth of pasture species under controlled environment. I. Growth at various levels of constant temperature. *N.Z. J. Sci. Tech.* A 38, 203—216.

—— and R. LUCANUS 1962 Growth of pasture species under controlled environment. III. Growth at various levels of constant temperature with 8 and 16 hours of uniform light per day. *N.Z. J. Agric. Res. 5,* 135—144.

NOWAKOWSKI, T. Z. 1962 Effects of nitrogen fertilizers on total nitrogen, soluble nitrogen and soluble carbohydrate contents of grass. *J. Agric. Sci. 59,* 387—392.

PLANK, J. E. VAN DER 1936 The estimation of sugars in the leaf of the mangold (*Beta vulgaris*). *Biochem. J.* No. 30, 457—483.

TROUGHTON, A. 1961 The effect of photoperiod and temperature on the relationship between the root and shoot systems of *Lolium perenne. J. Brit. Grassl. Soc. 16,* 291—295.