Reprinted from *Proceedings of the Eighth International Grassland Congress, 1960*

PRINTED IN GREAT BRITAIN

PAPER 8B/1

THE EFFECT OF NITRATE NUTRITION ON CARBOHYDRATE CONTENT IN *LOLIUM PERENNE*

TH. ALBERDA

(*Institute for Biological and Chemical Research on Field Crops and Herbage, Wageningen, The Netherlands*)

Summary. A clone of perennial ryegrass, *Lolium perenne*, was cultivated in different culture solutions under constant environmental conditions. Defoliation of the plants caused a temporary decrease in the total water-soluble carbohydrates (TSC), running parallel with a temporary increase in the nitrate content of the plant. When the plants were left intact while the nitrate concentration of the nutrient solution was increased, the same features were observed although nitrogen was not limiting growth during the experiment.

Although the decrease in TSC-content and the increase in nitrate content were greatest when defoliation coincided with a rise in the nitrate concentration of the solution, this did not result in a decrease in the rate of dry matter production as compared with plants where defoliation took place a week before or a week after the rise in nitrate concentration.

Résumé. Un clone d'ivraie vivace, *Lolium perenne*, a été cultivé dans différentes solutions de culture dans des conditions d'ambiance constantes. La défoliation des plantes provoqua une réduction temporaire de la teneur totale en glucides solubles dans l'eau, qui allait de pair avec une augmentation temporaire de la teneur de la plante en nitrates. Lorsque les plantes furent laissées intactes cependant que l'on accroissait le pourcentage de nitrates dans la solution nutritive, on observa les mêmes caractéristiques, quoique l'azote ne limitait pas la croissance durant l'expérience.

Malgré le fait que le baisse dans la teneur totale en glucides solubles et l'augmentation de la teneur en nitrates atteignaient un maximum lorsque la défoliation coïncidait avec une augmentation de la concentration de la solution en nitrates, ceci n'eut pas pour effet de diminuer le taux de production de matières sèches par comparaison avec les plantes où la défoliation survînt une semaine avant ou une semaine après l'augmentation dans la concentration en nitrates.

Zusammenfassung. Ein Klon von *Lolium perenne* ist in verschiedenen Nährlösungen unter gleichbleibenden Umgebungsbedingungen gezogen worden. Entblätterung der Pflanzen führte zu zeitweiliger Abnahme der Gesamtmenge der Wasser-löslichen Kohlehydrate (TSC), die parallel mit einer zeitweiligen Zunahme des Salpeter-Salz Inhaltes der Pflanze verlief. Wenn die Pflanzen intakt gelassen wurden, während die Nitrat-Konzentration der Nährlösung erhöht wurde, konnte man die gleichen Anzeichen beobachten, obwohl der Stickstoff das Wachstum während des Experiments nicht behinderte.

Obwohl die Abnahme des TSC-Gehalts und die Zunahme des Salpeter-Salz Inhalts ihren Höhepunkt dann erreichten, wenn die Entblätterung mit einer stärkeren Nitrat-Konzentration der Lösung zusammenfiel, führte dieses nicht zu geringerer Erzeugung von Trockenmasse im Vergleich zu Pflanzen, bei den die Entblätterung eine Wohce vor oder eine Woche nach der Erhöhung der Salpeter-Salz Konzentration vorgenommen wurde.

Introduction

It has been shown (1, 5) that the defoliation of grass causes a temporary slowing-down of the rate of dry matter production together with a reduction in carbohydrate content, especially in the stubble. This adverse effect of defoliation could be enhanced by both a high night temperature and a low light intensity. Harrison (3) has demonstrated that a high nitrogen fertilization may lead to a very severe reduction of grass production after repeated defoliation, as compared with a low N fertilization. The same trend has been found in field experiments. Mulder (4), for instance, showed that when a very high yield has been obtained by high N fertilization, aftermath growth will be rather poor. These facts have led to a closer study of the effect of N nutrition on the carbohydrate reserves, with and without cutting.

Experimental methods

The experiments were carried out in the phytotron of the Institute (2). The plant material consisted of a single clone of *Lolium perenne*. Selected tillers were placed in a Hoagland nutrient solution of half strength and cultivated in a glasshouse at 20° C until they had tillered and rooted sufficiently to use them in the experiments. All experiments were carried out in the growth rooms at a temperature of 20° C, a light intensity of 5×10^4 ergs. $cm^{-2}sec^{-1}$ and a daylength of 17 hours.

The nitrate concentrations used in the experiments will be indicated as the proportion of the nitrate concentration in the full-strength Hoagland solution. Consequently N 1/2 indicates the Hoagland half-strength solution used for normal growth; N 1/8 indicates that the nitrate concentration has been reduced by replacing part of the KNO_3 and $Ca(NO_3)_2$ by K_2SO_4 and $CaCl_2$, respectively; N 4/1 indicates that the nitrate concentration has been increased by adding the two nitrate salts to the half-strength solution to get the required concentration. In order to be sure that the concentrations were maintained at the appropriate level, the solutions were generally renewed daily.

Results

Three experiments were carried out. In the first the effect of cutting alone was considered, in the second the nitrate concentration in the solution was varied, while the third experiment was a combination of the first two.

In the first experiment the plants were grown on the normal half-strength solution (N 1/2) throughout. All plants were cut at a height of 5 cm several days after the beginning of the experiment. From then on plants were taken for analysis at suitable intervals.

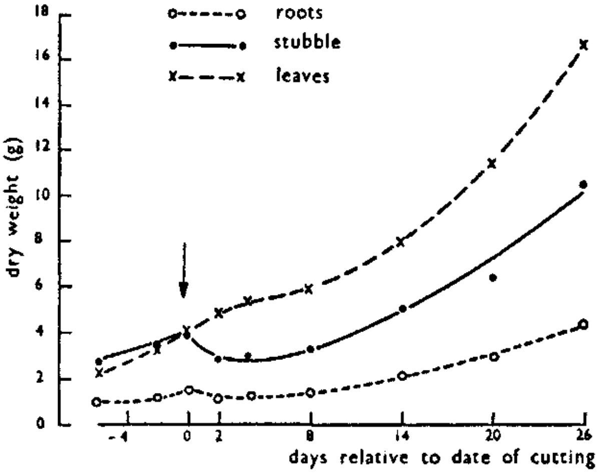

Fig. 1. Changes in the dry weight of roots, stubble and leaves; the arrow indicates the time of cutting.

Figure 1 gives the changes in the dry weight of roots, stubble (the basal 0–5 cm of the tillers) and leaves (the rest of the tillers) during the experiment. As has been found previously there was a small reduction in root weight just after cutting and a rather severe reduction in stubble weight. A reduction in the rate of leaf production

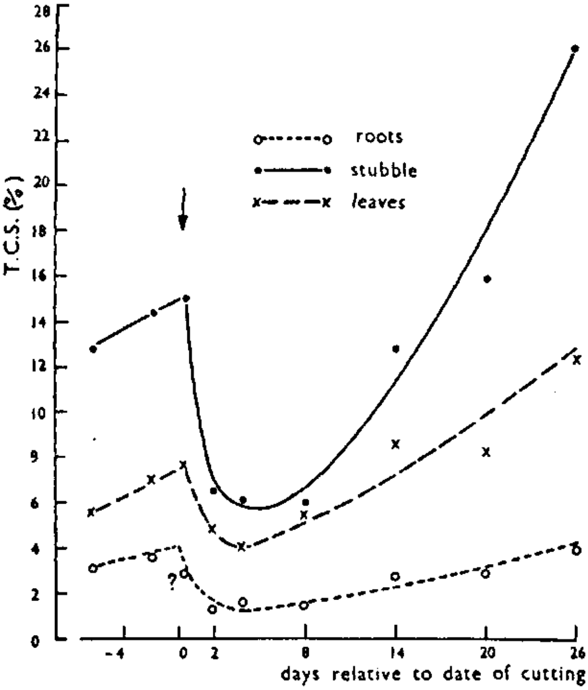

Fig. 2. The total water-soluble carbohydrate content in roots, stubble and leaves as a percentage of dry weight; the arrow indicates the time of cutting.

was not very pronounced. It must be mentioned that the weight of the cut-leaf material has been added to the subsequent leaf weights. After about a week both stubble and roots again increased in weight at an increasing rate.

Figure 2 shows the total water-soluble carbohydrates (= TSC; mainly fructosans) as a percentage of the dry

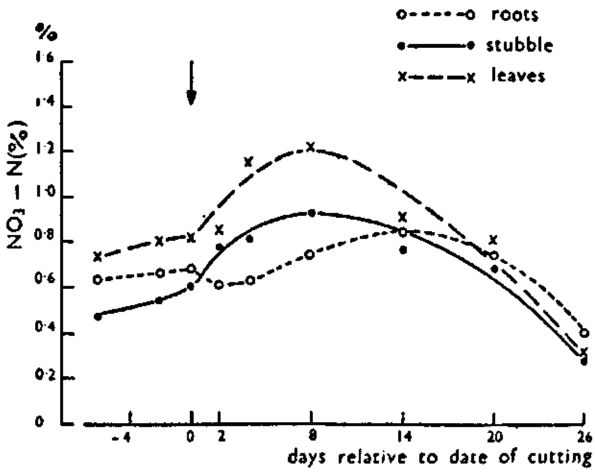

Fig. 3. The nitrate content in roots, stubble and leaves as percentage nitrogen in the dry weight; the arrow indicates the time of cutting.

weight. It appears that there was a considerable reduction in this percentage during the first 5 days after cutting, followed by a recovery.

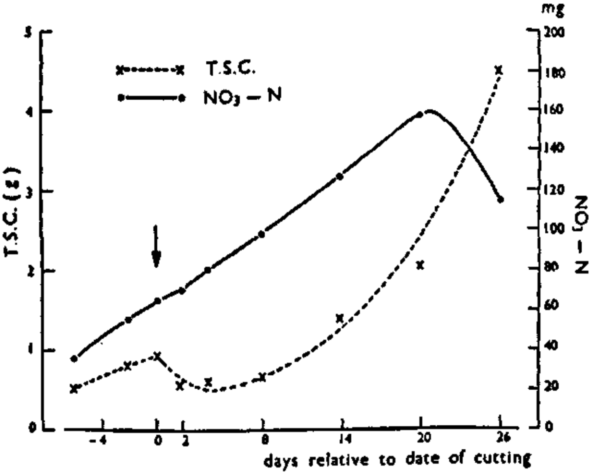

Fig. 4. The amount of total water-soluble carbohydrates and nitrate nitrogen in the whole plant; the arrow indicates the time of cutting.

Figure 3 gives the percentage nitrate nitrogen in the dry weight of the 3 portions of the plant. The trend after cutting was roughly the opposite to that of the TSC-content. The nitrate content rose rapidly in stubble and leaves during the first week after cutting and diminished gradually thereafter. The percentage in roots decreased slightly just after cutting, and then began to increase. The increase in the amount of nitrate in the whole plant was hardly influenced by cutting (Figure 4). Because of this it is uncertain whether the changes in percentage nitrate, observed in the 3 plant portions, were due to the decrease in the amount of dry matter only (Figure 5), or to changes in both the rates of nitrate uptake and of nitrate incorporation into organic form.

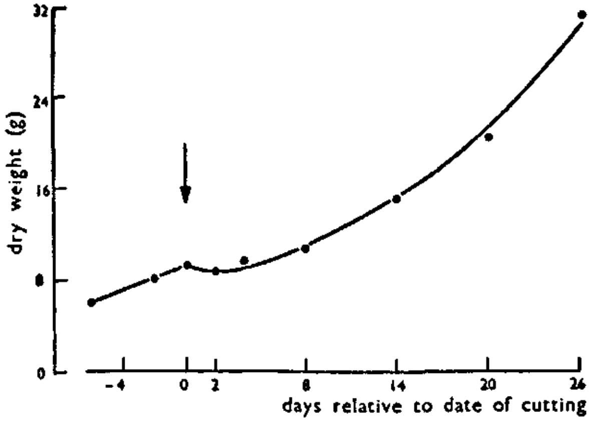

Fig. 5. The dry matter production of the whole plant; the arrow indicates the time of cutting.

The latter supposition seems to be the most probable since it was found earlier (unpublished) that the rate of nitrate uptake by roots decreased after cutting and in addition the amount of TSC in the whole plant decreased considerably as well (Figure 4). That the constant rate of increase of nitrate was more or less incidental also appeared from the next experiment, in which a gradual decrease in the amount of nitrate in the whole plant was followed by a sudden increase immediately after cutting.

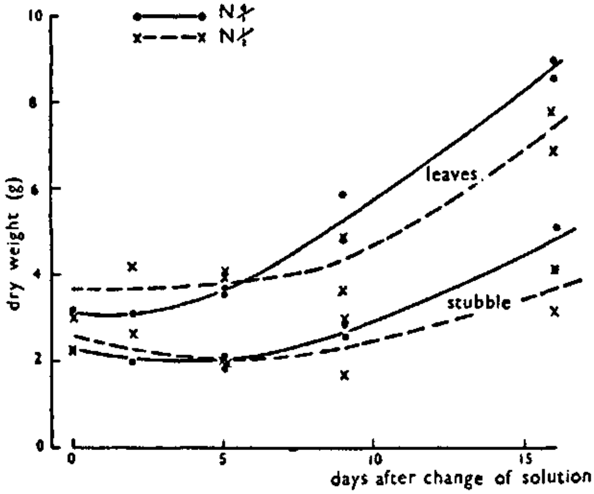

Fig. 6. Changes in the dry weight of stubble and leaves.

In the second experiment the plants were not defoliated. Two weeks before the experiment proper the plants were brought into the growth room and at the same time the N 1/2 solution was replaced by a N 1/8 solution. At the start of the experiment the plants were divided into 2 groups. From one of these groups the solution was brought back to N 1/2; the other group was given a N 4/1 solution. From the beginning of the experiment plants from both groups were taken for analysis at regular intervals.

Figure 6 gives the course of dry matter production of stubble and leaves. The general trend as to differences in dry weight and chemical composition of the roots was the same as for the other plant portions, and the data are not presented. The large difference in nitrate concentrations between the culture solutions resulted in only small differences in dry matter production. There was a slight indication of a reduction in stubble weight with both groups after the change in nitrate concentration.

When the TSC-content is expressed as a percentage of dry weight (Figure 7) it can be seen that this percentage in both stubble and leaves dropped considerably after the nutrient solution had been changed. The pattern was very similar to that found after cutting, and the percentage was considerably lower with the N 4/1 than with the N 1/2 concentration.

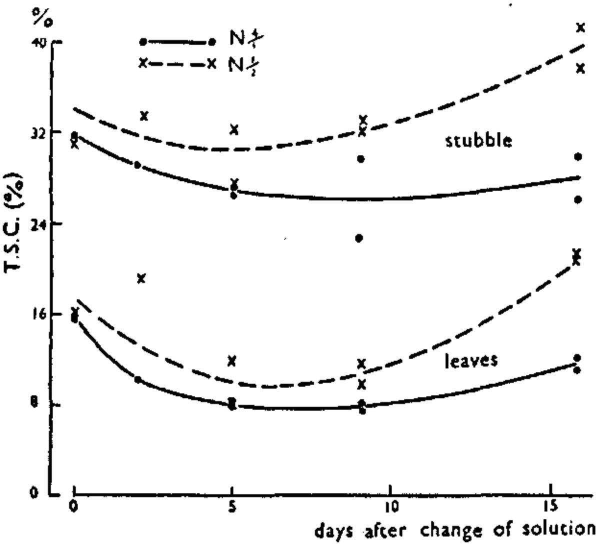

Fig. 7. The total water-soluble carbohydrates in stubble and leaves as a percentage of dry weight.

The nitrate content of both stubble and leaves (Figure 8) showed a great increase when the solution was changed from N 1/8 to N 4/1. The rate of increase was greatest during the first part of the experiment and slowed down gradually; in the leaves there seems to have been a slight decrease towards the end of the experiment. With a nitrate content N 1/2 in the culture solution, the increase was not nearly so large and in both stubble and leaf a decrease set in after about a week. At the end of the experiment the nitrate content was again at about the same low level as at the beginning.

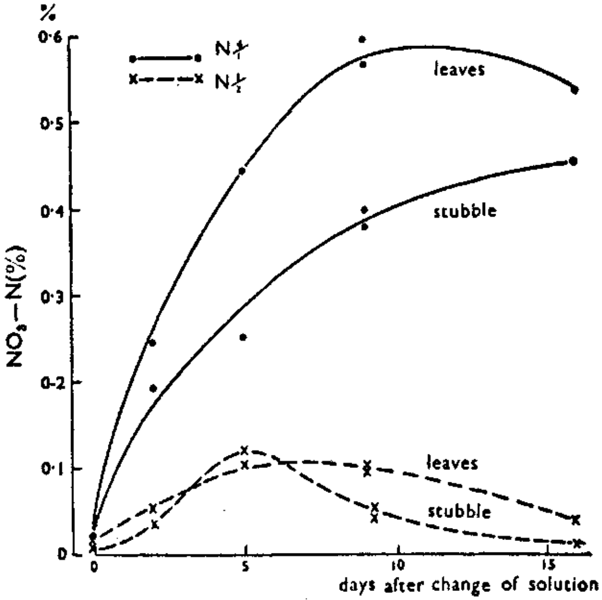

Fig. 8. The nitrate-nitrogen content of stubble and leaves as a percentage of dry weight.

Crude protein contents were not analysed in this experiment, but even without these data it can be concluded that a nitrate level in the culture solution which lies far above normal does not change the production of dry matter to a great extent but does change the chemical composition considerably.

Since it has been shown that both cutting and a change in nitrate concentration in the culture solution lower the TSC-content in the plant appreciably, one might question to what level the TSC-content of plants might drop when the nitrate concentration around the roots is raised at the moment of defoliation, as is usually done in grassland management.

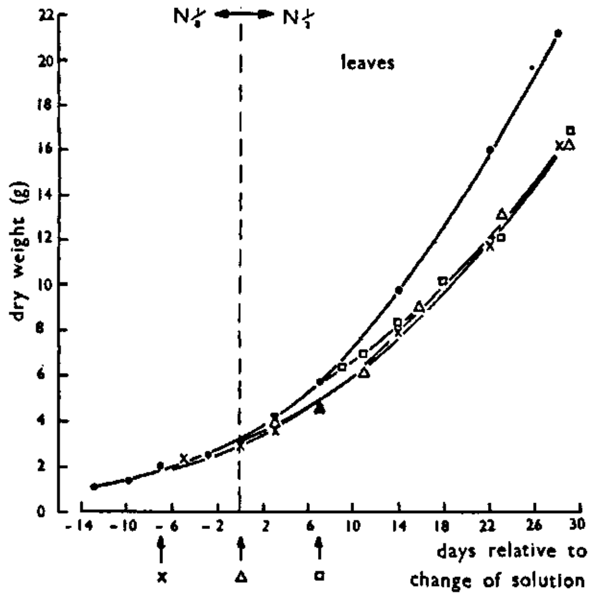

Fig. 9. Changes in leaf dry matter production of undefoliated plants and of plants defoliated at different times relative to the change in nitrate concentration; the arrows indicate the times of cutting (see text).

For this purpose a third experiment was carried out in which the plants were divided into 6 groups. All groups were cultivated on a N 1/8 solution until 10 days after the start of the experiment, when it was changed to a N 1/2 solution. One group of plants was left undefoliated, a second group was defoliated at the time the solution was changed; the other 4 groups were defoliated respectively 1 week before and after, and 3 days before and after, the change of solution.

Plants were taken at suitable intervals from all groups for analysis for dry weight, TSC- and nitrate-content. Only the data for leaves are given here since those for stubble and roots show very similar results.

Figure 9 gives the leaf dry matter production of 4 of the 6 treatments. It can be seen that the rate of leaf production was retarded after cutting, but for all cutting dates the rate was the same at the end of the experiment. Also if one compares total dry matter production the time of defoliation had no distinct influence. Therefore, it can be concluded that the coincidence of defoliation and higher N nutrition has no adverse influence on dry matter production. Apparently a nitrate concentration in the nutrient solution of one-eighth of normal is still sufficient for good growth provided that the solution is renewed daily. At any rate no change in growth rate could be observed in the undefoliated plants after the change from N 1/8 to N 1/2.

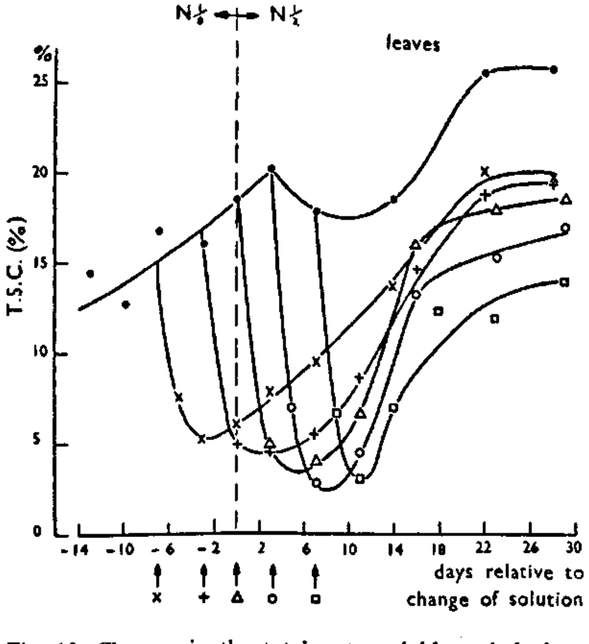

Fig. 10. Changes in the total water-soluble carbohydrate content as a percentage of dry weight, both in undefoliated plants and in plants defoliated at different times relative to the change in nitrate concentration; the arrows indicate the times of cutting (see text).

The course of the TSC-content of the leaves of all groups is given in Figure 10. The figures for stubble and roots showed the same general trend and are therefore omitted. It can be seen that the TSC-content of the uncut plants increased up to 3 days after the change of the nutrient solution from N 1/8 to N 1/2. The percentage then decreased for about 1 week, after which it again increased to a level, at the end of the experiment, of about 26% of the dry weight. The drop that occurred after cutting in the other groups was far steeper than the drop caused by the rise in N concentration alone, and greatest in the group that had been defoliated 3 days after the change of the culture solution. Not only did the TSC-content reach its peak value just before cutting but it also reached its lowest value just after cutting (about 2·5%). This represented a drop of more than 17% in 1 week.

In the stubble the greatest drop was found with the plants of the group defoliated 3 days before the change of the solution, viz. from 30 to 13%. The TSC-content of the uncut plants dropped immediately after the change of the solution but was found to be much less pronounced than in the cut groups (about 2% only).

With leaves the drop in TSC percentage was somewhat steeper and the recovery was more rapid when cutting had taken place at a later date. It seems that the later defoliation takes place the lower will be the ultimate level at the end of the experiment. However, the experiment was not continued long enough to state this with certainty.

This difference in the rate of change in the TSC-content at different cutting dates was not found in the stubble and, moreover, there was no indication that a definite level was reached at the end of the experiment.

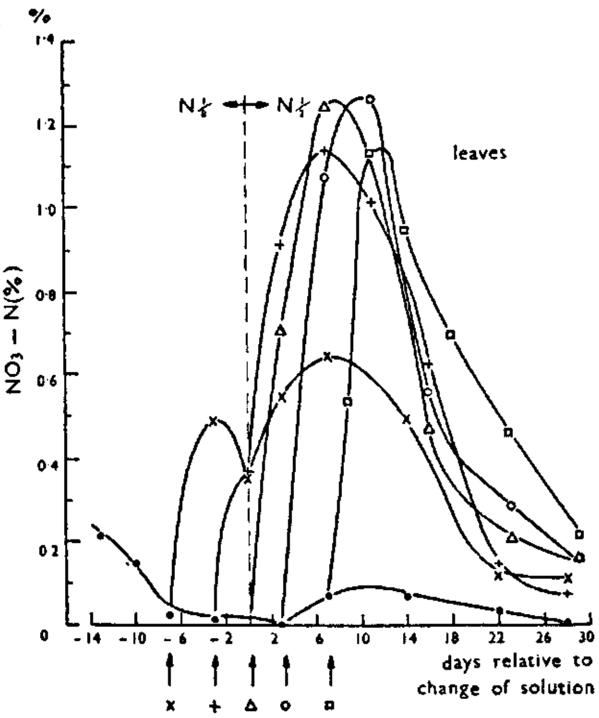

Fig. 11. Changes in the nitrate-nitrogen content in the leaves as a percentage of dry weight, both in undefoliated plants and in plants defoliated at different times relative to the changes in nitrate concentration; the arrows indicate the times of cutting (see text).

The nitrate content of the leaves of the uncut plants remained at a very low level throughout the experiment (Figure 11). At the time of changing the solution less than 0·02% N was present in the leaves in the nitrate form. Three days later no inorganic N at all could be detected. After that date the effect of changing the solution became visible. The nitrate content rose to about 1·3% in 1 week and decreased from then on to zero at the end of the experiment.

Defoliation had a very pronounced effect on inorganic-N content which rose after the first cut from the very low value of the uncut plants to about 0·5% in 4 days. Thereafter a decrease set in until the nitrate concentration in the solution was changed. This caused an increase to a higher value, viz. 0·6% N in inorganic form. This peak value lasted for a very short time only; soon the nitrate content dropped to a low value again. When cutting occurred 3 days before the change of the solution a 2-peaked curve was barely evident. Thus the behaviour of nitrate in the plant is just the opposite to that of the soluble carbohydrates. When cutting is given later the peak is sharper. The experiment has not been continued long enough to see if the same inorganic-N content will be reached for all cutting treatments. The curves for the nitrate content of stubble and roots were also the opposite of those of the TSC-content. The correlation between nitrate and TSC-content in the leaves was −0·95.

Discussion

These experiments have shown that cutting or increasing the nitrate concentration of the nutrient solution have comparable effects on the TSC- and inorganic-N content of plants. The TSC-content always drops considerably, especially in the stubble, and the nitrate content rises to rather high values.

The effect of cutting is usually of short duration; after about 3 weeks the original levels of both TSC- and nitrate-content are reached again. This appeared from both the first and third experiments. The second experiment, however, indicated that differences in nitrate concentration in the nutrient solution, which give almost no difference in dry matter production, nevertheless have a distinct influence on the chemical composition. The percentage of carbohydrates at the end of the experiment was distinctly higher with a N 1/2 solution than with a N 4/1 solution. This confirmed an earlier experiment (not made in the phytotron) in which plants were cultivated for prolonged periods at N 1/8 and N 1/1 concentrations. The leaves were cut 3 times at 3-weekly intervals. The difference in chemical composition between the 2 treatments increased with the successive cuts. At the end of the experiment the ratio TSC/crude protein was 1·45 with a N 1/8 solution and 0·17 with a N 1/1 solution. Further research on this subject is in progress.

The third experiment convincingly demonstrated that the coincidence of a sudden increase in the N-concentration around the roots and defoliation has no adverse effect on dry matter production as compared with defoliations before or after the high N-concentration. The changes in chemical composition were considerable but were of rather short duration. Consequently nitrate concentrations high enough to be poisonous to grazing cattle are unlikely, since a sward is not likely to be grazed again so soon after a defoliation.

BIBLIOGRAPHY

1. ALBERDA, TH. 1957. *Plant and Soil*, **8**, 199-230.
2. ALBERDA, TH. 1958. *Acta. bot. néerl.*, **7**, 265-77.
3. HARRISON, C. M. 1934. *Plant Physiol.*, **9**, 83-106.
4. MULDER, E. G. 1949. *Versl. Inst. landb. Onderz., Wageningen*, **55**, No. 7, pp. 55.
5. SULLIVAN, J. T. and SPRAGUE, V. G. 1943. *Plant Physiol.*, **18**, 656-70.