CLIMATE, NITROGEN AND GRASS

Research into the influence of light intensity, temperature, water supply and nitrogen on the production and chemical composition of grass

B. DEINUM

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CLIMATE, NITROGEN AND GRASS

Research into the influence of light intensity, temperature, water supply and nitrogen on the production and chemical composition of grass

(MET EEN NEDERLANDSE SAMENVATTING)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS, IR. F. HELLINGA,
HOOGLERAAR IN DE CULTUURTECHNIEK,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 2 DECEMBER 1966 TE 16.00 UUR

DOOR

B. DEINUM

H. VEENMAN & ZONEN N.V. – WAGENINGEN – 1966

STELLINGEN

I

De grenswaarde van 100 meq. NO_3 per kg droge stof, waarboven de drogestofopbrengst van een grasgewas niet meer verhoogd wordt door stikstofbemesting, geldt alleen bij een lichtsterkte van ca. 300 cal cm^{-2} dag^{-1}. Bij lagere lichtsterkte ligt de grenswaarde hoger, bij hogere lichtsterkte daarentegen lager.

Dit proefschrift
VAN BURG, P.F.J., Versl. landbouwk. Onderz. Ned. 1962, 68–12, 1–131

II

In tegenstelling tot de gedachte van ALBERDA, worden in gras de gehalten aan nitraat en aan wateroplosbaar koolhydraat vrijwel steeds onafhankelijk van elkaar beïnvloed door de uitwendige omstandigheden.

Dit proefschrift
ALBERDA, Th., Neth. J. agric. Sci. **13** (1965), 359

III

De geringe voederwaarde van het gras in de tropen wordt vooral veroorzaakt door de daar heersende hoge temperaturen.

Dit proefschrift

IV

De als verouderingsverschijnselen beschreven veranderingen in het ruweiwit- en ruwe-celstofgehalte tijdens de ongestoorde groei van een grasgewas zijn voornamelijk het gevolg van veranderingen van de drogestofopbrengst, de lichtsterkte en de temperatuur.

V

In tegenstelling tot de gangbare opvatting, wordt de opname aan verteerbare droge stof uit gras door de herkauwer veel sterker bepaald door zijn opname aan droge stof, dan door deszelfs verteerbaarheid.

MILFORD, R., Proc. 9th Intern. Grasslnd Congress, Sao Paulo 1965.

VI

Van de huidige meettechnieken betreffende de drogestofopname van weidend vee mogen slechts beperkte resultaten worden verwacht.

Proefschrift B. DEINUM

Wageningen, 2 december 1966.

VII

De in Nederland bij het routine-onderzoek gebruikelijke techniek voor het bepalen van de zetmeelwaarde van gras en grasprodukten voldoet redelijk voor de praktijk, maar bij het wetenschappelijk onderzoek is zij veelal onvoldoende nauwkeurig. Daarom moet zo spoedig mogelijk getracht worden, betere technieken te ontwikkelen of uit het buitenland te introduceren.

VIII

Door zijn betere verteringskapaciteiten kunnen in de tropen iets hogere dierlijke produkties verwacht worden van de *Bos indicus* dan van de *Bos taurus.*

HOWES, J. R., J. anim. Sci. **22** (1963), 20–26.

IX

Bij veredeling op de kwaliteit van gras en andere voedergewassen is het zinvoller te selekteren op de verteerbaarheid, dan op het gehalte aan ruw eiwit, wateroplosbaar koolhydraat en dergelijke.

LACKAMP, J. W., Versl. landbouwk. Onderz. Ned. 1965, no. 656, 1–120

X

De fotosynthese-snelheid van gewassen wordt zo sterk bepaald door de grootte van het bladoppervlak, dat het noodzakelijk is om in proeven waarin de invloed van diverse faktoren op de fotosynthese wordt onderzocht, beide grootheden steeds in hun onderlinge samenhang te bestuderen.

WATSON, D. J., Rothamsted Report 1965, 92.

XI

In tegenstelling tot de meest gebruikelijke procedure, verdient het aanbeveling tijdens grote internationale kongressen, onderwerpen, waarover meerdere sprekers een bijdrage willen leveren, slechts te laten inleiden door één of twee experts, en de overigen de gelegenheid te geven, deel te nemen aan de diskussie.

CONTENTS

1. INTRODUCTION 1

2. REVIEW OF THE LITERATURE 2
2.1. Constituents investigated 2
2.2. Factors affecting the dry-matter production and chemical composition of grass . 3
2.2.1. Aging 3
2.2.2. Season 4
2.2.3. Nitrogen fertilization 6
2.2.4. Water supply 7
2.2.5. Temperature 7
2.2.6. Light intensity 9

3. PLANT PHYSIOLOGICAL BACKGROUNDS 10
3.1. Production of dry matter 10
3.2. Chemical composition 13
3.2.1. Nitrate 14
3.2.2. Nitrogen 15
3.2.3. Ash 17
3.2.4. Water-soluble carbohydrate 17
3.2.5. Crude fibre and rest 18

4. LAY OUT OF THE EXPERIMENTS 20
4.1. Meteorological data 20
4.2. Indoor experiments 20
4.2.1. Indoor experiments 1963 20
4.2.2. Indoor experiment 1964 24
4.3. Field experiments 24
4.3.1. Field experiments 1962 and 1963 24
4.3.1.1. Field experiments 1962 25
4.3.1.2. Field experiments 1963 25
4.3.2. Field experiment, spring 1964 26
4.3.3. Field experiment, autumn 1964 26
4.3.4. Twenty-five years' experiment 1938–1962 27
4.4. Method of collecting and treating the grass 27

5. RESULTS AND DISCUSSION OF INDOOR EXPERIMENTS 29
5.1. Indoor experiment 1963-III 29
5.1.1. Influence of light intensity 29
5.1.2. Influence of temperature 30
5.1.3. Influence of water supply 32
5.1.4. Interaction of light intensity and water supply 32
5.2. Indoor experiment 1964 33
5.2.1. Influence of light intensity 33
5.2.2. Influence of fertilization 33
5.2.3. Interaction of light intensity and fertilization 35
5.3. Comparison of the results of the various indoor experiments 36
5.3.1. Level differences between the experiments 36
5.3.2. Influence of light intensity 40
5.3.3. Influence of temperature 41
5.3.4. Influence of water supply 43
5.3.5. Interaction of light intensity and water supply 44

5.4. Discussion . . . 44
5.4.1. Dry-matter production . . . 44
5.4.2. Nitrate . . . 44
5.4.3. Crude protein . . . 45
5.4.4. Ash . . . 47
5.4.5. Water-soluble carbohydrate . . . 47
5.4.6. Crude fibre . . . 49
5.4.7. Rest . . . 53

6. RESULTS OF THE FIELD EXPERIMENTS . . . 55
6.1. Field experiment, spring 1964 . . . 55
6.2. Field experiment, autumn 1964 . . . 56
6.3. Field experiments 1962 and 1963 . . . 58
6.3.1. Influence of nitrogen fertilization . . . 58
6.3.2. Influence of water shortage . . . 59
6.3.3. Influence of light intensity and temperature . . . 62
6.4. Twenty-five years' experiment 1938–1962 . . . 63
6.4.1. Influence of light intensity and temperature on the dry-matter production and chemical composition of grass in all cuts together . . . 64
6.4.2. Influence of light intensity and temperature on the dry-matter production and chemical composition of grass in the separate cuts . . . 65
6.4.2.1. Influence of light intensity . . . 65
6.4.2.2. Influence of temperature . . . 65
6.4.2.3. Differences between the cuts . . . 67
6.4.3. Comparison of the light and temperature effect on the grass per cut to that on the entire material . . . 67

7. GENERAL DISCUSSION AND CONCLUSIONS . . . 69
7.1. Comparison of all the experimental results . . . 69
7.1.1. Dry-matter production . . . 69
7.1.2. Nitrate . . . 69
7.1.3. Crude protein . . . 70
7.1.4. Ash . . . 70
7.1.5. Water-soluble carbohydrate . . . 70
7.1.6. Crude fibre . . . 71
7.1.7. Rest . . . 71
7.1.8. Nitrogen-free extract . . . 72
7.2. Influence of the growth rhythm of the grass on the seasonal trend in the dry-matterproduction and the chemical composition . . . 72
7.3. Comparison of the chemical composition of Dutch grass and tropical grass . . . 75

SUMMARY . . . 77

ACKNOWLEDGEMENT . . . 79

SAMENVATTING . . . 80

REFERENCES . . . 82

APPENDIX . . . 87

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1. INTRODUCTION

The importance of grass is determined for a great part by its nutritive value for cattle and sheep.

In the Netherlands this nutritional value is expressed in the digestible crude-protein content (% dcp) and starch equivalent (SE) of the forage. The direct determination of these quantities is difficult and very expensive, therefore they are inferred from the chemical composition. Already in 1860 HENNEBERG et al. developed a method to determine the chemical composition and up to the present day this so-called Weende system has been used to determine the nutritive value of grass and other forages in various countries.

It goes without saying that since 1860 much research has been carried out into the factors affecting the chemical composition and with it the nutritive value of the herbage. Some of the most important factors found are: grass species, the age of the grass, season and nitrogen fertilization (e.g. BROWN, 1943; VAN BURG, 1962; MINSON, 1964). The nutritive value of old grass as a rule is lower than that of young grass; in the same way the nutritional value of autumn grass is lower than that of spring grass in the Netherlands. Nitrogen fertilization affects the starch value of young grass favourably, but that of old grass unfavourably. The nutritive value of perennial ryegrass is higher than that of cocksfoot in the same growth stage.

Beside the above-mentioned research, plant physiologists investigated the substances formed in the growing plant under varying conditions (e.g. LEOPOLD, 1964). Frequently the same chemical constituents are investigated as in the fodder research, e.g. protein and nitrate, various carbohydrates, cellulose and lignin. In these experiments often controlled conditions of light, temperature and mineral supply were maintained.

In the present investigation grass was grown under more or less controlled conditions to study the influence of light intensity, temperature, water and nitrogen supply on the dry-matter production and chemical composition. In the chemical analysis methods were applied, adjusted to those used in the fodder research. In this way the quantitative influence of these factors on the grass plant could be investigated as well as their effect on the nutritive value.

Firstly a survey of the literature is given concerning the factors which influence the chemical composition of grass, as well as a possible explanation of these effects. Next the effect of the various factors is treated in detail in the light of the results of this research, attempting to quantify the effects. This research is centred on the habitat factors, leaving the age and genetic characters of the grass out of consideration.

Indoor as well as field experiments were carried out in this investigation. In the indoor experiments the effects of the climatological factors light and temperature could be separately studied as well as those of the water supply and fertilization. The field experiments were set out to investigate if these factors are active also under natural conditions and to what extent their effects correspond in indoor and field experiments.

2. REVIEW OF THE LITERATURE

2.1. CONSTITUENTS INVESTIGATED

The first point of discussion is the dry-matter yield. This indicates the dry-matter yield of the cut grass except for a few cases in which the dry-matter yields of the stubble and roots were also determined.

In chapter 1 the Weende system has already been mentioned. In this method the dry matter is divided into:

crude protein	(cp)
ash	(ash)
crude fibre	(cf)
nitrogen-free extract	(nfe)

The nitrogen-free extract is not directly determined, but calculated from 100 − % cp − % cf − % ash.

Crude protein consists of proteins, amides, amino-acids and nitrate. Its content is determined by multiplying by 6.25 the nitrogen content according to KJELDAHL. In the analysis a part of the nitrate volatilizes. DIJKSHOORN (1960) found this was 15–60%. In the Netherlands the nitrate content is usually low under practical conditions. Under certain conditions, however, high nitrate contents may occur. The nitrate-free protein content is most frequently applied in this investigation.

Ash is the total complex of minerals remaining after ashing the dry matter. Crude fibre is a most complicated group of compounds, insoluble in acid and hydroxide.

The digestible crude-protein content is calculated from the crude-protein and ash content. The starch equivalent is inferred from the crude-fibre and ash content. In the Netherlands regression equations, developed by DIJKSTRA (1957) with the results of digestion trials with wethers, are applied in this. The calculated starch equivalent is not sufficiently accurate for the present purpose and therefore only the contents of the chemical constituents are mentioned in this publication.

In this investigation the water-soluble-carbohydrate content (% wsc = mono- + disaccharides + fructosans) and the nitrate content have been added to the chemical composition according to HENNEBERG et al. The fructosans are polymers of fructose with a chain to about 50 fructose-units (SCHLUBACH et al., 1957).

A non-determined rest remains after these five analyses. This 'rest' content cannot be determined, but is always calculated from % r = 100 − % NO_3 − % cp − % ash − % wsc − % cf. However, the analysis errors of the five preceding constituents accumulate in this rest and must be considered in studying this content.

If the chemical composition of the dry matter is determined and the different constituents are expressed in percentages of the dry matter the sum of % cp, % cf, % ash, % wsc, % NO_3 and % r must always be 100. If one or more contents increase, affected by certain conditions, this implies, that the other contents decrease.

2.2 Factors affecting the dry-matter production and chemical composition of grass

It is well known that the contents of the before-mentioned constituents may vary considerably in grass from different origins. The botanical composition, physiological age of the material and the growing conditions are important factors in this. The following factors will be subsequently treated: aging, season, nitrogen fertilization, water supply, temperature and light.

2.2.1. *Aging*

The external conditions permitting, grass will grow. If these conditions are favourable the dry-matter yield increases so long as the herbage is allowed to grow, thus with the aging of the grass. With this rising dry-matter yield its chemical composition will also change; this process has frequently been described in the literature, e.g. by van Burg (1962), Kivimae (1959). An example taken from Frankena (1941), is mentioned in table 1.

Table 1. Influence of the length of the growing period on the dry-matter production and chemical composition of grass (in percentages of the dry matter) (Frankena, 1941)

Date	kg dm ha^{-1}	% cp	% ash	% cf	% nfe
12/V /1934	3420	18.7	10.9	26.1	44.3
19/V /1934	4030	15.8	10.8	25.5	48.2
26/V /1934	5750	14.3	9.3	27.6	48.2
5/VI/1934	6160	11.9	9.1	30.8	48.2
15/VI/1934	7790	10.6	8.6	33.1	47.7

In aging grass, the crude-protein and ash contents decrease, and the crude-fibre content increases. The nitrogen-free-extract content is lower on the first harvest date of the experiment than on the following dates.

Without nitrogen application the young crop will show a low nitrate content, which will decrease only slightly with the aging of the crop (van Burg, 1962). If a liberal nitrogen fertilization is applied the nitrate content in the young grass is high but will sharply decrease when the crop matures (van Burg, 1962; 1965).

The water-soluble-carbohydrate content does not show a distinct trend. Jones (1962) found in different varieties of perennial ryegrass a low content in young grass, which initially increased with aging, but which was reduced later. Waite et al. (1953[I]) mentioned a similar trend in perennial ryegrass and cocksfoot. The changes in the water-soluble-carbohydrate content of the grass were considerable, as shown in table 2.

The nitrogen fertilization in this experiment was rather low (about 30 kg N/ha), which also led to high sugar contents in this spring and summer grass. Furthermore, the water-soluble-carbohydrate content in cocksfoot is much lower than in perennial ryegrass. Waite et al. suggested that the decrease in perennial ryegrass after 10 June is caused by the development of the stem for which

TABLE 2. Influence of the length of the growing period on the water-soluble-carbohydrate content of two grass species (WAITE et al., 1953[1])

Average sampling date	Perennial ryegrass	Cocksfoot
28/ IV/1952	9	8
10/ V/1952	20	10
25/ V/1952	23	10
10/ VI/1952	33	15
28/ VI/1952	27	16
10/VII/1952	28	–
28/VII/1952	25	–
31/VII/1952	24	12

much carbohydrate is needed. BROWN et al. (1963) found a similar trend in the autumn grass. ALBERDA (1963), however, mentions a rising content in aging grass.

The changes in the dry-matter yield and in the contents of all the constituents are shown again in table 3 (data DEINUM, 1964). This table again clearly reflects the mentioned aging trends.

TABLE 3. Influence of the length of the growing period on the dry-matter production and chemical composition of spring grass
Fertilization: 100 kg N/ha (DEINUM, 1964)

Date	kg dm ha^{-1}	% dm	% NO_3	% cp	% ash	% wsc	% cf	% r
24/IV/1963	560	14.9	–	33.9	9.5	6.5	17.7	32.6
1/ V/1963	1070	14.0	0.70	28.6	10.2	8.1	19.8	32.6
8/ V/1963	1920	16.0	0.18	20.4	8.3	18.2	18.9	34.2
22/ V/1963	3460	14.6	0.14	14.5	7.3	18.0	22.5	37.9
5/VI/1963	5810	21.0	0.06	9.5	5.8	16.9	27.9	40.0
19/VI/1963	7200	15.8	0.04	7.7	5.7	11.4	33.2	41.8

2.2.2. *Season*

Age symptoms are found in grass which may grow undisturbed. After cutting, grass will show fresh growth from the stubble. A certain periodicity may be found in the grass production and chemical composition, when the grass is cut at regular intervals. JAGTENBERG (1961) mentions experiments in which grass was cut every five weeks. This periodicity is shown in table 4 which mentions the average dry-matter yields per ha and per day and the crude-protein content of grass in the grassland area of Friesland during a period of 13 years.

After an adequate production in spring the dry-matter yield of the grass is depressed in summer. This is called the mid-summer depression. In autumn the grass growth decreases rather rapidly until the production almost terminates as a result of unfavourable weather conditions.

The crude-protein content is initially high, decreases rather rapidly in early summer, increasing again slowly to a maximum in autumn.

Seasonal curves may also be calculated from KLETER'S (1961) data. In his

TABLE 4. Seasonal trend in the dry-matter production and crude-protein content in 5-weeks' old grass (JAGTENBERG, 1961)

Average harvest date	kg dm $ha^{-1}day^{-1}$	% cp
5/V	56.3	19.3
10/VI	62.2	15.4
15/VII	43.8	17.1
20/VIII	44.8	18.5
25/IX	28.0	20.3
1/XI	4.2	21.6

investigation the grass was harvested and sampled at a dry-matter yield of about 2000 kg/ha and not at the same age. Table 5 shows the average dry-matter yields and the contents arranged per month (data of 1957).

TABLE 5. Dry-matter yield and chemical composition of grass in different months of the year (KLETER, 1961)

	kg dm ha^{-1}	% cp	% ash	% cf	% nfe
May	2211	17.1	11.8	23.5	47.6
June	2160	16.0	10.6	25.4	48.0
July	1953	17.4	11.5	26.5	44.6
Aug.	2201	18.2	12.4	26.0	43.4
Sept.	1859	19.4	13.9	25.4	41.3

It can be seen that the seasonal trend in the crude-protein content has almost disappeared with the same dry-matter yields in the various months. The ash content shows a fair increase during the summer. This was probably caused by contamination of the grass with soil particles. The crude-fibre content is low in spring and high in summer; in autumn there is a tendency to decrease. The nitrogen-free-extract content is highest in spring and gradually decreases afterwards.

BROWN (1943) found a similar trend as JAGTENBERG in the crude-protein content of *Poa pratensis* of 14 days old. In spring and autumn the crude-fibre content was lowest (about 18–20%) and it was highest in summer (about 24%). The nitrogen-free-extract content did not show a distinct trend.

WAITE et al. (1953[II]) found in experiments, corresponding with those of KLETER, a high water-soluble-carbohydrate content in spring (up to about 25%), a low one in summer (2–5%) and a somewhat increased content in autumn. However, considerable fluctuations may occur in this general trend. ALBERDA (1955) mentions a similar trend; he considers these fluctuations are caused by the time of sampling and by the grassland management.

Some data are known on the trend in the nitrate content. The content is very low in spring and summer, but in late autumn some nitrate may be found in the grass (MULDER, 1949). In practice this content is so low, that it is of little importance in the chemical composition and nutritional value of herbage.

2.2.3. *Nitrogen fertilization*

The effect of nitrogen on grass production and chemical composition has already been frequently investigated. VAN BURG (1962) summarized the elaborate literature on this subject. Under Dutch conditions the effect of the nitrogen in practice is as follows. Nitrogen fertilization will raise the dry-matter yield considerably even to such an extent, that it shows a greater yield-increasing effect than all other factors up till now. The increase in the dry matter may achieve 40 kg dm $kg^{-1}N$ (FRANKENA, 1941). The effect depends on:

a. the rate of the nitrogen application. With very high applications the effect will be less as a result of the diminishing returns (VAN BURG, 1960; FRANKENA, 1941);
b. the growing period (VAN BURG, 1960; FRANKENA, 1941). The longer the growing period the larger the nitrogen effect;
c. the season (VAN BURG, 1960; OOSTENDORP, 1964). In spring the effect is larger than in summer and autumn;
d. the nitrogen mobilization in the soil. On a nitrogen rich soil the effect will be less than on a nitrogen poor soil (FRANKENA, 1941);
e. the drainage. Poorly drained soils will show a smaller nitrogen effect (MINDERHOUD, 1960);
f. the water supply. Water deficiency will also decrease the nitrogen effect (VAN BURG, 1962).

Nitrogen fertilization also considerably affects the chemical composition. The dry-matter content is usually reduced by nitrogen fertilization. However, it will frequently increase the nitrate content in the grass. VAN BURG (1962) considers the correlation between the nitrate content in the grass and the nitrogen supply to the plant close enough to apply the nitrate content in the plant as a standard for its nitrogen supply. With a nitrate content over 0.6% the supply of additional nitrogen is supposed not to affect the dry-matter production; below this level it does. ALBERDA et al. (1962) mention this level as well.

The crude-protein content is frequently increased by nitrogen fertilization... This increase depends on the fertilization level and on the length of the growing period. Shortly after the fertilization this rise is considerable; as the growing period is longer the increase will be smaller. After long growing periods the contents in the fertilized and non-fertilized treatments may even be the same (VAN BURG, 1962; FRANKENA, 1941; MULDER, 1949). Under Dutch conditions with a growing period of four weeks the increase is significant with a nitrogen fertilization higher than about 60 kg N/ha/year.

Few experiments have been made on the influence of nitrogen fertilization on the ash content. FRANKENA (1941) mentions sometimes an increase and sometimes a decrease. The water-soluble-carbohydrate content is considerably diminished by nitrogen fertilization (ALBERDA, 1959; 1960; 1965).

It is generally assumed, that the crude-fibre content is increased by nitrogen fertilization. This was investigated in detail by VAN BURG (1962). Based on the results of his experiments he states that in long growing periods the crude-fibre content is increased. In a short growing period, however, it does not increase and a decrease may even be found. However, when VAN BURG compares the crude-fibre contents with equal dry-matter production, he always finds a decrease in the crude-fibre content by nitrogen fertilization. He suggests this is caused by a rejuvenating effect of nitrogen on grass.

The rest content is reduced by nitrogen fertilization (ALBERDA, 1965). This is reflected in table 6, which also shows the effect of nitrogen fertilization on the dry-matter production and the contents of all the other constituents in the first cut (data DEINUM, 1964).

TABLE 6. Influence of the nitrogen fertilization on the dry-matter production and chemical composition of grass
(harvest date: 22/V/1963) (DEINUM, 1964)

N-dressing kg ha^{-1}	kg dm ha^{-1}	% dm	% NO_3	% cp	% ash	% wsc	% cf	% r
0	680	21.1	0.05	9.6	5.3	27.3	18.7	39.1
50	2400	17.0	0.05	10.9	5.9	25.3	20.1	37,3
100	3460	14.6	0.15	14.5	7.3	18.0	22.5	37.9
200	4260	12.8	0.97	19.8	7.5	14.3	23.4	35.2

2.2.4. *Water supply*

Water shortage always retards the dry-matter production (VAN BURG, 1962; NIELSEN, 1963), and the dry-matter content being rather high.

In one of the experiments of VAN BURG (1962), carried out under extremely dry conditions, the nitrate content was usually somewhat lower. He mentions, however, various authors finding increased nitrate contents under drought conditions.

Sometimes the crude-protein content is reduced (VAN BURG, 1962), sometimes it is higher (VAN BURG, 1962; MAKKINK, 1960; VAN RIPER, 1964). VAN BURG (1962) states that the nitrogen uptake is inhibited in periods of extreme and lasting drought, which leads to low nitrate and crude-protein contents, in less extreme and shorter periods of drought the nitrogen uptake should be less hampered, resulting in higher nitrate and crude-protein contents.

Not much has been published on the influence of water shortage on the ash content. Like the nitrate and crude-protein contents the water-soluble-carbohydrate content is also differently affected. ARCHBOLD (1938) mentions reduced contents with drought, whereas WEINMANN (1948) and SHAPIRO et al. (1963) refer to increased contents. The extent of the water shortage might be important also in this case (TROUGHTON, 1957).

VAN BURG (1962) states that the crude-fibre content is reduced by drought. The same can be inferred from the work of 'T HART (1960), in which he found a negative correlation between the starch value of hay and silage and the precipitation minus evapotranspiration of the growing period.

2.2.5. *Temperature*

On earth plant growth is possible in a rather wide temperature range. Each plant species has a minimum and a maximum temperature; at these temperatures growth is just possible. If *Lolium perenne* may grow undisturbed the optimum temperature is between 20 and 25°C (ALBERDA, 1957; DEL POZO, 1963). This was found in the first cut of an experiment; if the grass was regularly cut the optimum temperature dropped to about 15°C after two cuts (DEL POZO, 1963).

The temperature also affects the chemical composition. With a liberal nitrogen supply the temperature usually has a positive influence on the nitrate content (ALBERDA 1965; BATHURST et al., 1958; BEEVERS et al., 1964). BROWN (1939) found the lowest crude-protein contents in various grass species at the optimum temperature and somewhat higher contents at lower and higher temperatures. The findings of BATHURST et al. (1958) correspond fairly well, although the differences are not considerable. ALBERDA (1965) found increased total nitrogen contents at higher temperatures, but the nitrate-free crude-protein content was only slightly affected by temperature. His experiments show that the ash content increases with the temperature.

The water-soluble-carbohydrate content is frequently diminished by temperature (ALBERDA, 1959; 1965; BATHURST et al., 1958; BEEVERS et al., 1964). The crude-fibre content is usually increased at higher temperatures (ALBERDA, 1965; BROWN, 1939; REDER, 1954).

Only ALBERDA (1965) has published about the rest content; it increases at a higher temperature. Table 7 (from ALBERDA, 1965) shows the effect of temperature on the various constituents.

TABLE 7. Influence of the temperature on the dry-matter production and chemical composition of the leaves of *Lolium perenne* in a light intensity of 5×10^4 erg $cm^{-2}sec^{-1}$ (ALBERDA, 1965)

Temperature °C	g dm	% NO_3	% cp[1]	% ash	% wsc	% cf	% r
10	13.6	0.58	17.8	9.0	33.4	14.7	24.5
15	23.1	1.11	20.9	10.0	20.3	17.7	30.0
20	29.4	1.37	20.8	11.2	15.5	19.5	31.6
25	32.8	1.20	23.5	11.2	10.8	21.2	32.1
30	19.6	1.68	22.4	14.0	7.4	22.0	32.5

[1] NO_3-free

The different trend in the day and night temperature is especially interesting. ALBERDA (1957; 1959) found in his experiments, that the dry-matter production is increased at a lower night temperature. DEL POZO (1963) mentioned the same. The water-soluble-carbohydrate content is also increased at a lower night temperature (BEEVERS et al., 1964).

Related to the mineral uptake, the root temperature is important. GROBBELAAR (1963) found in maize at the optimum root temperature the lowest dry-matter content, the highest contents of the various minerals and the lowest water-soluble-carbohydrate content in the overground parts.

2.2.6. *Light intensity*

Experiments in which the effect of light intensity was investigated always showed, that a higher light intensity resulted in an increased dry-matter production (e.g. ALBERDA, 1957; 1965; ARTHUR, 1930; BURTON et al., 1959).

The chemical composition is also affected by the light intensity. Thus the nitrate content is usually decreased at a higher light intensity (e.g. ALBERDA, 1965; BURSTRÖM, 1943; CRAWFORD et al., 1961), as well as the crude-protein content (e.g. ARTHUR, 1930; BATHURST et al., 1958; BLACKMAN et al., 1940; BURTON et al., 1959). However, ALBERDA'S (1965) findings showed a decreased total nitrogen content with a liberal nitrogen supply at a higher light intensity, but there was no influence of the light intensity on the nitrate-free crude-protein content.

At a higher light intensity the ash content is usually decreased (ALBERDA, 1965). TINCKER (1928) mentions a low content with a long day. A long day may have the same effect as a high light intensity, because in both cases the total amount of light supplied per day may be high.

Many authors found, that the water-soluble-carbohydrate content was increased at higher light intensities (e.g. ALBERDA, 1957; 1965; BATHURST et al., 1958; BURTON et al., 1959).

The crude-fibre content is usually decreased at higher light intensities (ALBERDA, 1965; JULEN, 1955). An increased crude-fibre content is seldom found (ROBERTS, 1926, cited by HOPPER et al., 1930). In the experiments of ALBERDA (1965) the rest content was not influenced by the light intensity.

The effect of light intensity cannot be easily expressed in absolute figures, because in most experiments the relative figures of light intensity are given, and not the absolute ones.

Finally the influence of light intensity on the chemical composition of the grass is shown in table 8 (from data by ALBERDA, 1965).

TABLE 8. Influence of the light intensity on the dry-matter production and chemical composition of grass (ALBERDA, 1965)

Light intensity (erg $cm^{-2}sec^{-1}$)	g dm	% NO_3	% cp[1]	% ash	% wsc	% cf	% r
2×10^4	18.0	2.35	20.0	13.8	11.6	22.0	30.2
5×10^4	23.7	1.20	21.1	11.1	17.5	19.0	30.1

[1] NO_3-free

3. PLANT PHYSIOLOGICAL BACKGROUNDS

The previous chapter treated the applied agricultural investigations into the influence of the various factors on the dry-matter production and chemical composition of grass, without mentioning the plant physiological backgrounds. These are, however, of great importance in understanding and explaining the mentioned effects. Therefore this chapter deals with these backgrounds.

Autotroph plants need for growth and production: CO_2, light energy, water and minerals, while the temperature must be within a certain range.

It is necessary to know to what extent these factors influence the dry-matter production. However, in cultivated crops the total dry-matter production is not the sole issue, the yield of the parts of economic value (e.g. seeds, fruits, roots, fibres) is especially interesting. In grass the overground parts to be mown or to be grazed are of direct importance.

The plant physiology is also significant in explaining the chemical composition of the grass. Since the contents of the various constituents in the plant are the result of various processes occurring in the plant, it is important to go into these processes.

The following processes will be successively treated; those leading to the production of dry matter and those leading to the contents of the various constituents in the dry matter.

3.1. Production of dry matter

The dry-matter yield actually is the difference between the CO_2-uptake and the CO_2-release, supplemented by water and minerals from the soil. In the presence of light the plant takes up CO_2; CO_2 is lost again by respiration. The following reactions take place in a simplified presentation:

$$6\ CO_2 + 6\ H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6\ O_2 \text{ (photosynthesis)}$$

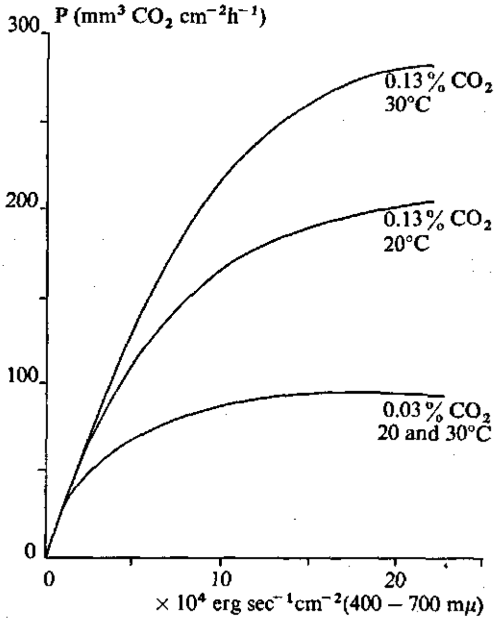
$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O \text{ (respiration)}$$

In the photosynthesis carbohydrate is formed via intermediate products; a part is lost again by respiration.

Thus CO_2, light energy and water are needed in photosynthesis. A quantitative interpretation of the influence of the CO_2-concentration in the air, light intensity and temperature on photosynthesis was put forward by GAASTRA (1962) (fig. 1). The plant can only assimilate CO_2, if light can be intercepted. Therefore light intensity often is the limiting factor in photosynthesis. Light saturation was attained in cucumber at a light intensity of about 0.3 cal $cm^{-2}min^{-1}$, the light rays hitting the leaf surface perpendicularly and with normal CO_2-contents of the air.

In the Netherlands this light intensity of 0.3 cal $cm^{-2}min^{-1}$ corresponds with

FIG. 1
Photosynthesis (P) of a cucumber leaf in relation to light intensity and temperature at a limiting (0.03%) and a saturating (0.13%) CO_2-concentration (after GAASTRA, 1962)



25–30% of bright sunshine on a clear day in June. Higher photosynthesis could only be achieved by increasing the CO_2-content in the air.

Photosynthesis to some extent is also influenced by the temperature (e.g. STALFELT, 1960). Each plant species is supposed to have its own optimum temperature in this. GAASTRA (1962) does not mention any difference in photosynthesis between 15–30°C with normal CO_2-contents in the air. With higher CO_2-contents photosynthesis was higher at 30°C than at 20°C (fig. 1).

Water shortage has a limiting effect on photosynthesis (BAKER et al., 1964).

VAN DER PAAUW (1956) and WATSON (1956) assume that photosynthesis is limited by a serious mineral deficiency. This is tenable on theoretical grounds, but both authors also mention, that this retardation is only observed occassionally in the many experiments carried out.

Respiration increases exponentially to the temperature, viz. per rise of 10°C (= Q_{10}) with a factor of 1.7–2.0 (STALFELT, 1960). According to GAASTRA there is hardly any increase in the gross photosynthesis with temperature, and according to STALFELT only a slight one. Therefore this appreciable temperature effect on respiration implies a more distinct optimum temperature of the net photosynthesis (= gross photosynthesis – respiration), than of the gross photosynthesis. Moreover, this optimum temperature is lower than that of the gross photosynthesis. The optimum temperature is also lower at lower light intensities (ALBERDA, 1965). This was also found in the experiments of MITCHELL et al. (1962) with various grass and clover species; however, they did not vary the light intensity, but the daylength, by which they obtained differences in the total light supplied per day.

These processes occur in the individual plant as well as in a crop. Nevertheless, it is important whether the plant has more or less space available. A solitary plant has adequate space overground as well as underground. The leaves on the plant are usually arranged in such a way, that each leaf intercepts all the light possible. In a crop there is, however, competition for overground and underground space. The light can be intercepted from above, and water and minerals are taken up from the soil, but only in that part of the space available to the plant.

Only the top leaves in a crop receive full day light. About 10% of the light is transmitted by a leaf, but the light transmission by the top layer of leaves may increase appreciably by the spatial arrangement and position of the leaves. BROUGHAM (1956) found in grass a light transmission of about 75% by the top layer of leaves, while at a leaf area index (LAI) of 5 about 5% of the light still reached the soil.

The intensity of the light falling on the lowest leaves is already very low and photosynthesis also. If the LAI is over 5 the lower leaves receive almost no light and therefore they are photosynthetically inactive. The whole plant respires, including the lower leaves; therefore the latter consume carbohydrates (DAVIDSON et al., 1958). The dry-matter production per day could be larger if these leaves were not present. This situation holds with normal light intensities. With higher light intensities the light penetrates deeper into the crop and the compensation point (net photosynthesis = 0) is lower. This implies that in this case the lower leaves may contribute to a small extent in the dry-matter production. With lower light intensities the compensation point is higher; in this case the lower leaves only respire. Since photosynthesis is much lower with low light intensities and because of the small number of leaves receiving sufficient light, the dry-matter increase per day with low light intensities is much lower than with high light intensities. This is reflected in fig. 2, taken from BLACK (1963), in which the dry-matter production in *Trifolium subterraneum* L. per unit soil area and per day has been plotted against the leaf area index at different light intensities.

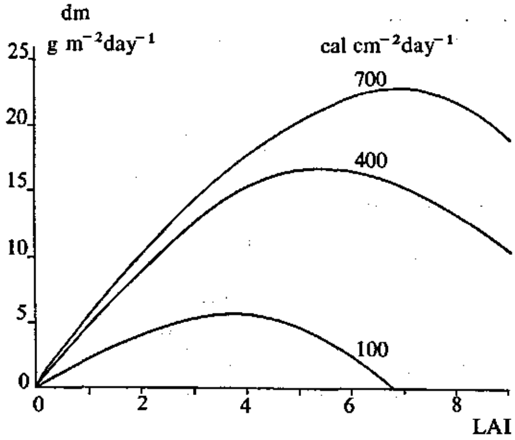


FIG. 2
The interrelationship of dry-matter increase, leaf area index (LAI) and light intensity for 'Bacchus March' subterranean clover (after BLACK, 1963)

This would imply, that in autumn, when the light intensity is low, the grass should be cut at a lower LAI and therefore at a lower dry-matter yield than in spring.

The total dry-matter production is not the only essential, but the distribution of the dry matter over the various organs is important as well. The total amount of material harvested is of primary importance in grass. The adequate formation of roots and stubbles is a second requirement. Nevertheless these organs are important in the regrowth.

In the first part of the life cycle of the plant, during the vegetative phase, the shoot/root-ratio is low; a relatively great part of the dry matter is transported to the root. When the plant moves into the reproductive phase, almost all assimilates remain in the overground parts and the root weight increases only slightly, whereas the shoot weight increases rapidly. Therefore the shoot/root-ratio rises sharply (BROUWER, 1962[I]). The stubble weight is influenced in a similar way as the root weight (SULLIVAN et al., 1949).

The dry-matter distribution is also affected by weather and fertilization conditions. TROUGHTON (1960[I] and [II]) described these influences. A high light intensity stimulates root growth relatively more than shoot growth; nitrogen fertilization has a reverse effect. Water shortage will retard shoot growth more than root growth. From BROWN'S (1939) experiments it can be calculated that the shoot/root-ratio increases with the temperature. However, in the experiments of ALBERDA (1965) and TROUGHTON (1961) this influence is not clearly evident.

BROUWER (1962[II]) and TROUGHTON (1960[II]) state, following LOOMIS, that the distribution of dry matter is a result of the competition for nutrients. Root growth is determined by the carbohydrate supply, shoot growth by the water and mineral supply: water and mineral deficiency will retard shoot growth, carbohydrate deficiency hampers root growth. The plant should aim at a balance between photosynthesis and mineral and water uptake, and with it at a constant C/N-ratio.

The regrowth of grass after cutting is also important. This regrowth to a large extent takes place at the expense of the reserves in stubble and root, which mainly consist of carbohydrates. It may be inferred from the literature that factors causing a high stubble and root production also promote the build-up of reserves and regrowth (DAVIES, 1965; DEL POZO, 1963).

3.2. CHEMICAL COMPOSITION

The chemical constituents to be treated may be divided into two groups:

a. constituents, mainly present in the cell contents (nitrate, crude protein, ash and water-soluble carbohydrates);
b. constituents, mainly present in the cell wall (crude fibre, and rest).

The constituents will be treated in the above-mentioned order.

3.2.1. *Nitrate*

Nitrate is a nutrient for the plant. The plant takes up nitrogen from the root medium, preferably as nitrate, but nitrogen can also be taken up as ammonia. The nitrate absorbed is used in the plant. The nitrate content is the difference per weight unit between the nitrate taken up and that consumed.

Nitrate uptake of course depends on the nitrate supply in the soil and its availability. The latter probably depends on the temperature (ALBERDA, 1965; GROBBELAAR, 1963) and on the water supply (VAN BURG, 1962).

Before the plant can use the nitrate, it has to be reduced to ammonia. Afterwards it is used in the synthesis of amino-acids and proteins. H^+-ions are needed in the nitrate reduction. These H^+-ions are formed in the oxidation of sugars, but especially in the photochemical separation of water in H^+ and OH^-. In the latter process many H^+-ions are formed under influence of the light. The following may be concluded from the literature on nitrate reduction (BONGERS, 1956; 1958; KESSLER, 1964; SPENCER, 1958).

a. Nitrate reduction takes place at the expense of the sugars in the dark. Reduction will also be possible in the presence of light.
b. The rate of photochemical separation of water in H^+ and OH^- depends on the light intensity. H^+-ions may be used in the CO_2-reduction (photosynthesis) as well as in the nitrate reduction. With low light intensity CO_2- and nitrate reduction are competitive with regard to the H^+-ions. With high light intensity the H^+-formation may furthei increase in the range where the CO_2-supply is the limiting factor for photosynthesis. Then there will be an excess of H^+-ions, resulting in a considerable nitrate reduction. Of course the maximum H^+-production depends on the amount of enzyme present.
c. There is a similar saturation curve for the nitrate reduction, influenced by the light intensity, as for the CO_2-reduction, because the nitrate supply cannot be raised unlimited.

This means:

d. In the dark the nitrate reduction depends on the concentration of water-soluble carbohydrates. If this concentration is low, nitrate is not reduced (ALBERDA, 1965).
e. With low light intensity CO_2- as well as nitrate reduction takes place. It may depend on the conditions which of the two is more in evidence. The C/N-quotient required by the plant might be important in this (BONGERS, 1956).
f. Photosynthesis as well as nitrate reduction are considerable with high light intensity and under favourable growing conditions. Even with a liberal nitrogen supply, the nitrate content in the plant may be low then.

Water deficiency will retard photosynthesis, mainly because the CO_2-uptake of the plant is hampered. Maybe a C-containing substance, formed during photosynthesis, is needed in reducing nitrate to nitrite (KESSLER, 1964). A deficiency of this substance might limit the nitrate reduction in drought.

The correctness of the mentioned effect of the various factors on the nitrate content as well as the extent of their influence will be investigated in the experiments described in chapter 4.

3.2.2. *Nitrogen*

The crude-protein content is a converted nitrogen content. In plant physiology the nitrogen content is used, in animal nutrition the crude-protein content. In this it is assumed that the crude protein always contains 16% of nitrogen.

The nitrogen is taken up as nitrate or ammonia from the soil and distributed in the whole plant. The nitrogen content in the dry matter of the plant is the quotient of the nitrogen uptake and the dry-matter production:

$$\% N = \frac{\text{N-uptake} \times 100}{\text{dry-matter yield}}$$

It is a well-known fact, that when excess nitrogen is available the plant will saturate itself with it, thus achieving a maximum nitrogen content. The nitrogen quantity in the plant is then related to the plant volume and with it more or less to the dry weight.

The trend in the nitrogen content, influenced by light intensity, temperature and water-supply is shown in figure 3. Figure 3a represents a schematic plotting of the dry-matter production, nitrogen uptake and time of a rapidly as well as a slowly growing plant. In figure 3b the nitrogen content has been plotted against time. The higher or lower production of both plants is the result of different light, temperature or water-supply treatments.

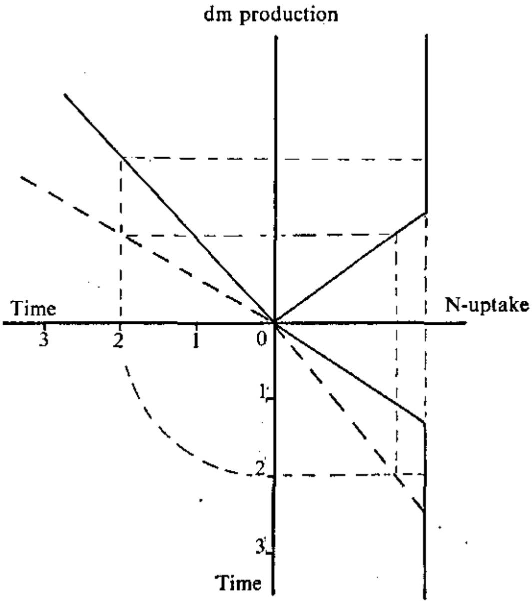


FIG. 3a
Schematic relationship of dry-matter production, nitrogen uptake and time of a rapidly (——) and a slowly (- - - - -) growing plant

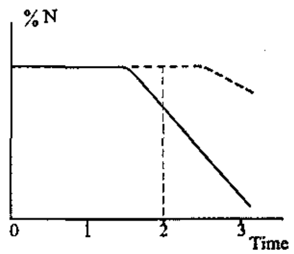


FIG. 3b
Schematic relationship between nitrogen content and time of a rapidly (——) and a slowly (- - - -) growing plant

At moment t_1, relatively short after the beginning of growth, the rapidly growing grass has produced more dry matter and has taken up more nitrogen than the slowly growing grass. An excess of nitrogen is still available for both plants. The nitrogen uptake is proportional to the dry-matter production, resulting in the nitrogen content remaining maximal. The external growth factors influence the dry-matter production and nitrogen uptake at this moment, but do not yet affect the nitrogen content.

At moment t_2 the rapidly growing grass has already depleted the nitrogen available and the nitrogen content is somewhat decreased: the slowly growing grass, however, has not formed sufficient dry matter yet to deplete the available nitrogen. The nitrogen content is therefore still maximal. In this case the external growth factors affect the dry-matter production as well as the nitrogen uptake and the nitrogen content. The moment at which the nitrogen content is reduced is achieved earlier in the rapidly than in the slowly growing plant.

At moment t_3 the rapidly growing as well as the slowly growing grass have produced sufficient dry matter to deplete the available nitrogen and the nitrogen content is reduced. This decrease is much larger in the rapidly growing grass than in the slowly growing grass. The external conditions still influence the dry-matter production, but no longer the nitrogen uptake. Via the dry-matter production they considerably affect the nitrogen content.

This general and very schematic presentation is based on the following assumptions:

a. The dry-matter production increases directly proportional to time. If this is not the case the preceding diagram remains the same, because the nitrogen uptake reacts in the same way as the dry-matter production.

b. The maximum nitrogen content in the plant is always the same. However, it is known that the maximum nitrogen content in the leaf sheath and stem is lower than that in the leaf blade. If the percentage of leaf blade decreases with time, as in a closed crop, the maximum nitrogen content will decrease too.

c. The nitrogen present in the soil is directly available like in a nutrient solution. The availability of the soil nitrogen, however, probably depends on the quantity present and its mobility. This implies that the curves in figures 3a and 3b are less rectilinear than indicated.

d. Light intensity, temperature and water supply affect the nitrogen uptake via the dry-matter production. In addition, they do not directly influence the nitrogen uptake much. However, the plant can take up the nitrogen less easy at extremely low and high temperatures than at optimal temperature. Water shortage may also hamper the nitrogen uptake.

It is pointed out that the picture presented holds with all nitrogen fertilizations. The nitrogen application also has a considerable positive effect on the dry-matter production. The moment of nitrogen depletion could be advanced because of this influence on the dry-matter production, but the larger nitrogen supply usually retards it.

In the stage of nitrogen depletion light intensity and temperature affect the nitrogen content as a result of their influence on the dry-matter production.

When this dry-matter production is included in the calculations the light and temperature effect disappears.

In the preceding the nitrogen content in the whole plant has been discussed. If, however, the nitrogen content in the various parts of the plant (e.g. overground parts of the grass) is considered, a direct light and temperature effect may be established, for the distribution of dry matter is affected by light intensity and temperature. Thus the dry-matter yield of the root is stimulated relatively more by light than the dry-matter yield of the shoot. Then a larger share of the nitrogen taken up is fixed in the root, and less nitrogen is available for the overground parts. With nitrogen exhaustion and high light intensity the nitrogen content in the overground parts is lower than might be expected from the dry-matter yield. At higher temperatures a smaller part of the dry matter is usually stored in the roots and in this case the nitrogen content in the overground parts can be higher than might be expected from the dry-matter yield.

Considering these points a clear effect of the factors light intensity, temperature, water-supply and nitrogen fertilization may be indicated, but not the extent of their influence on the nitrogen content and with it on the crude-protein content.

3.2.3. *Ash*

The ash contains all the mineral elements remaining after ashing the sample. These minerals, like nitrogen, are taken up from the soil and distributed over the whole plant. The ash content may therefore be calculated in the same way as the nitrogen content:

$$\% \text{ ash} = \frac{\text{ash quantity} \times 100}{\text{dry-matter yield}}$$

In general the ash content is affected in the same way as the nitrogen content by the factors light intensity, temperature, water supply and nitrogen application.

3.2.4. *Water-soluble carbohydrate*

The water-soluble carbohydrates function in the plant as an intermediate product. Carbohydrates are formed, but also consumed. The water-soluble-carbohydrate content is the difference per weight unit between the formation and consumption of carbohydrates. Water-soluble carbohydrates in grasses from temperate regions consist of fructose, glucose, sucrose and fructosans.

In paragraph 3.1. it has already been mentioned that the carbohydrates are formed during photosynthesis. Photosynthesis increases with the light intensity, as does the quantity of water-soluble carbohydrates.

The carbohydrates are used in various processes, such as the formation of proteins and new tissues, respiration, while another part is accumulated in the storage organs.

At low temperatures the carbohydrate consumption for the respiration and formation of tissues is low, whereas the carbohydrate formation by photosynthesis still is adequate. Therefore the water-soluble-carbohydrate content will be high. As mentioned in paragraph 3.1. the respiration increases exponentially with temperature, therefore the carbohydrate loss by respiration will also increase exponentially. The dry-matter production is largest at the optimum temperature; it may be assumed, that the largest amount of sugars is then converted to substances needed in the formation of tissues. At the optimum temperature therefore the water-soluble-carbohydrate content will be low. At temperatures above the optimum, respiration will increase, but the formation of tissues decreases; if the combined carbohydrate consumption by respiration and tissue formation should be reduced the carbohydrate content could increase again. ALBERDA (personal information) indeed found this at one time.

The soluble carbohydrates are also used in the protein formation. Nitrogen is required for this. The protein formation increases with the nitrogen uptake of the plant. Since the nitrogen supply also affects growth, increased nitrogen applications reduce the carbohydrate content by protein as well as tissue formation.

Water shortage will hamper photosynthesis and with it the carbohydrate formation. The tissue formation is also retarded and sometimes the nitrogen uptake too. Therefore it will depend on the conditions if the water-soluble-carbohydrate content is decreased or increased by water shortage.

The above mentioned shows that occassionally the qualitative influence of external conditions may be established for the water-soluble-carbohydrate content, but their quantitative effect is not known.

3.2.5. *Crude fibre and rest*

The constituents treated in the preceding paragraphs are mainly found in the cell contents. The crude fibre and so-called rest are formed by the cell walls, fats and organic acids. The cell walls mainly consist of pectin, cellulose, lignin and hemicellulose. Since the parenchyma cells usually are thin-walled the amount of cell-wall material in this tissue is relatively small. In grass considerable amounts of cell wall are found in the vascular bundles and supporting tissues (e.g. HESSING, 1922).

Investigations of GAILLARD (1964) show that crude fibre is composed of about 90% of the cellulose, about 25% of the lignin and about 25% of the hemicellulose. The remainder of the cellulose, lignin and hemicellulose together with all pectin is recovered in the rest fraction.

Few papers have been published about the influence of external conditions on the amount of cell wall.

The crude-fibre and rest content, in fact, refer to the amount of cell wall in relation to the total dry-matter yield. In the preceding it was disclosed, that the crude-protein, ash and water-soluble-carbohydrate contents were low at an optimal temperature; this implies that the crude-fibre and rest content together must be high. Furthermore, photosynthesis and with it the carbohydrate formation will be high in high light intensity. The cell contents mainly consist of carbohydrates then. In view of this the amount of cell wall in relation to the total dry matter may decrease in increasing light intensity.

There is little more to be said about the trend in the crude-fibre and rest contents. The investigations to be treated will show the direction in which the various factors act and the extent of their influence.

The chapters 2 and 3 show that rather thorough qualitative investigations have been carried out on the effect of external conditions on the various constituents of the dry matter. Since interactions continuously take place between the contents of the separate constituents, quantitative figures of the contents in the grass grown under certain conditions can as yet not be predicted from these investigations.

In the experiments described a quantitative determination of these relations has been attempted from a number of well defined growing conditions. It has also been attempted to gain some more detailed idea about the plant physiological backgrounds.

4. LAY OUT OF THE EXPERIMENTS

The experiments already mentioned in the previous chapters were carried out in the years 1962, 1963 and 1964. Indoor as well as field experiments were concerned, for the greater part carried out in the greenhouses and on the experimental fields of the Department for Field Crops and Grassland Husbandry of the Agricultural University at Wageningen.

In the indoor experiments the light intensity, temperature, fertilization and water supply could be separately varied. In the field experiments, on the other hand, light intensity, temperature and water supply usually are fixed quantities, besides being correlated to some extent. This rather complicates a quantification of these influences; regression calculations may enable this.

However, with special arrangements the light intensity and water supply in the field may be varied; the fertilization can easily be controlled. The possibility to vary these three factors in the field was of course utilized.

The following paragraphs deal with the description of the meteorological data on the experimental years, and the lay out of the indoor and field experiments.

4.1. METEOROLOGICAL DATA

These data on 1962, 1963 and 1964, like the many-years' averages, have been mentioned as monthly averages in figure 4. In as far as possible data were applied on our own experimental fields or those of the meteorological station at Wageningen (0.5 km from the laboratory). If these data were not available those of the meteorological station at De Bilt (35 km from Wageningen) were used. In general the average weather conditions at Wageningen and De Bilt correspond closely, so that it is justified to use the many-years' averages of De Bilt at Wageningen.

Precipitation data on the three experimental years were recorded at the author's laboratory; those of the total global radiation, expressed in cal $cm^{-2}day^{-1}$, were recorded at the meteorological station at Wageningen; those of the 24-hours' temperature, least dependent on local conditions, were recorded at De Bilt.

In treating the results of the long lasting experiment, mentioned in paragraph 4.3.4., the data were used of the meteorological station at Eelde (about 20 km from the experimental field and about 150 km from Wageningen). The total global radiation was not measured at this station, but calculated from the percentage of bright sunshine according to DE BOER (1961).

4.2. INDOOR EXPERIMENTS

4.2.1. *Indoor experiments 1963*

These experiments were set out to determine the influence of light intensity,

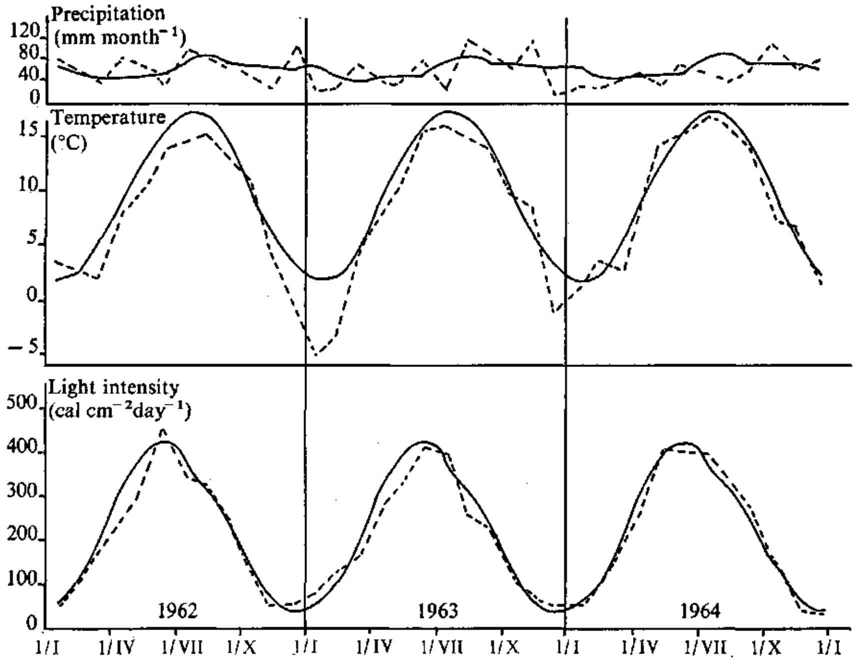


FIG. 4 Meteorological data on 1962, 1963 and 1964, as well as the many-years' averages (plotted as monthly averages)
—— Mean precipitation, temperature or light intensity
- - - - - Actual precipitation, temperature or light intensity

temperature and water supply on grass at different times of the growing season.

In November 1962, 700 5-litre plastic pots were filled with 4.75 kg humus and nutrient rich sandy soil, mixed with some peat dust, and planted with 31 seedlings of *Lolium perenne*, pasture type, var. Mommersteeg's. In January 1963, after the plants had established somewhat, 600 pots were placed in a greenhouse at 2°C to be vernalized. The remaining 100 pots were placed in a greenhouse at 15°C and a daylength of 15 hours to prevent vernalization.

On 19 March the 600 pots were brought outside. The grass was harvested every four weeks and fertilized with the nutrient solution, as applied to experiment 1963-II (table 12). In this way the usual management of the grass during the season was copied. In each experiment over 90 pots were used. The characteristics of the grass in the various experiments are mentioned in table 9.

It would have been satisfactory if the experiment 1963-II and -III could have been carried out under the same conditions at the same time for comparison of vegetative and shooting grass, but separation was inevitable because of the lack of space in the greenhouses.

During the experiments usually three light intensities, three temperatures and two moisture levels were applied in the greenhouses. The following temperatures

TABLE 9. Characteristics of the grass in the different parts of the indoor experiments 1963

Experiment	Experimental period	Vernalized or not	Shooting or not
1963- I	22/ III–19/ IV	vernalized	not
1963- II	19/ IV–17/ V	not	not
1963-III	17/ V–14/ VI	vernalized	shooting
1963-IV	14/ VI–12/ VII	vernalized	somewhat
1963- V	12/VII– 9/VIII	vernalized	somewhat
1963-VI	9/ X– 5/ XI	vernalized	not

were applied: 15–10°C, 20–15°C and 25–20°C (day and night temperature respectively). The night temperature was taken 5°C lower to be more within keeping with the normal 24-hours' trend in the outside temperature. The day temperature was applied between 7.00 a.m. and 7.00 p.m., the night temperature between 7.00 p.m. and 7.00 a.m.. The temperatures mentioned in table 10 were actually recorded by a thermograph (average per experiment).

TABLE 10. Actual temperatures in the different parts of the indoor experiments 1963

Experiment	t_1		t_2		t_3	
	day	*night*	*day*	*night*	*day*	*night*
1963- I	15.5	10.0	19.8	14.0	26.2	19.3
1963- II	15.8	9.9	20.2	13.7	26.5	20.0
1963-III	16.3	9.3	21.5	14.9	24.9	19.4
1963-IV	15.2	8.3	21.0	14.6	24.1	18.7
1963- V	17.7	8.6	22.6	14.8	25.4	19.0
1963-VI	15.0	9.1	19.9	13.7	24.5	18.7

Besides a natural light intensity, the plants were subjected to a high and a low light intensity compared to normal day light. The light intensity is expressed in cal $cm^{-2}day^{-1}$, measured with a Kipp's solarimeter, recording the natural light intensity. The figures reflect the global radiation (ca. 300–3,000 mμ). About 40% of this is in the visible region (400–700 mμ) (DE WIT, 1959). The high light intensity was obtained by additional light application with Philips HPL-400W lamps.

The spectral composition of the HPL-light and sunlight is not the same. In the calculations the intensity of the visible light of the HPL-lamps (measured with a standardized selenium cell) was multiplied by 100:40 to obtain the same standard as for the global radiation. The intensity of both kinds of light can be added in that case. The HPL-light was applied from sunrise until sunset, so that the daylength in the experiments was always the same as the natural daylength. The advantage of applying additional light during the day is, that high light intensities may be achieved.

The natural illumination in the greenhouses at the temperature t_2 and t_3 was about 80% of the natural day light, that in the greenhouse at t_1 about 75%. The low light intensity was obtained by shading with cheese cloth. The light intensities obtained are mentioned in table 11 (averaged per experiment).

TABLE 11. Light intensity in the various treatments in cal $cm^{-2}day^{-1}$, averaged per experiment (indoor experiments 1963)

Experiment	t_1			t_2, t_3			In the field
	L_1	L_2	L_3	L_1	L_2	L_3	
1963- I	42	167	293	51	178	303	222
1963- II	61	240	382	74	256	399	320
1963-III	80	317	472	97	338	493	422
1963-IV	64	253	410	78	270	427	337
1963- V[1]	–	301	449	–	321	467	400
1963-VI	21	83	167	25	88	173	110

[1] In experiment 1963-V only the treatments t_1, $L_2 + L_3$; t_2L_2 and t_3, $L_2 + L_3$ were present

It can be seen that very high light intensities were achieved in these experiments. In four experiments of the six an intensity was attained at the highest level about equal to or higher than that in June in the field.

The following moisture levels were applied:

moist: the soil was approximately kept at field capacity, so that the grass always had sufficient water available;

dry: the water supply was limited to such an extent, that the average water consumption was about 30% of the moist treatments.

Each treatment consisted of 5 pots.

Fertilization was applied by nutrient solutions; the quantities, expressed in meq./pot, are mentioned in table 12.

TABLE 12. Mineral application per experiment in meq./pot (indoor experiments 1963)

Experiment	N	K	Mg	H_2PO_4	SO_4
1963- I	16	8.5	8	2.5	14
1963- II	32	8.5	8	2.5	14
1963-III	48	8.5	8	2.5	14
1963-IV	48	8.5	8	2.5	14
1963- V	64	17	16	5	28
1963-VI	48[1]	8.5	8	2.5	14

[1] Dry treatments 32 meq./pot

Experiment 1963-I was used to try out the technique and to study the behaviour of the plants. It was found that the nitrogen requirement of the grass in the pots was considerably higher than anticipated. Therefore the nitrogen application in the next experiments was increased and adjusted to the dry-matter production to be expected. The nitrogen was applied as NH_4NO_3 in three to four doses to prevent salt damage.

At the end of an experiment the grass was harvested. The following was determined:

initial weight of roots and stubbles (2–5 pots per experiment);
dry-matter production and content;
chemical composition;
ultimate weight of roots and stubbles (1 pot per treatment).

4.2.2. *Indoor experiment 1964*

Fertilization levels were not applied in the indoor experiments of 1963. Because it is important to know the effect of fertilization on the dry-matter production and chemical composition, also in relation to climatical influences, in 1964 an experiment was started in which the fertilization and light intensity were varied.

In January 1964 about 50 pots were filled with 6 kg humus and nutrient rich sandy soil and planted with 31 seedlings of *Lolium perenne*, pasture type, var. Mommersteeg's. Until 24 April the plants were grown in a greenhouse at a temperature of 15° C and a daylenght of 15 hours to prevent vernalization. The experiment lasted from 24 April to 22 May 1964. The temperature in the greenhouse was 16.7 °C during the day and 10.5 °C during the night.

The light levels were obtained in the same way as in 1963 (see paragraph 4.2.1.). The light intensities per day in the experiment averaged:

L_1	91 cal $cm^{-2}day^{-1}$
L_2	263 cal $cm^{-2}day^{-1}$
L_3	538 cal $cm^{-2}day^{-1}$
field	351 cal $cm^{-2}day^{-1}$

The fertilization levels are mentioned in table 13.

TABLE 13. Fertilizations applied in meq./pot in indoor experiment 1964

Treatment	N	K	Mg	H_2PO_4	SO_4	Ca	Cl
N_1	8	0	0	0	0	0	0
N_2	32.5	8.5	8	2.5	14	0	0
N_3	97.5	17	16	17	16	9	9

Each treatment consisted of 5 pots. The water supply was arranged in the same way as in the moist treatments in the indoor experiments of 1963. The following data were determined:
dry-matter production and content;
chemical composition.

4.3. FIELD EXPERIMENTS

4.3.1. *Field experiments 1962 and 1963*

The purpose of these experiments was to investigate the influence of light intensity, temperature, water supply and nitrogen fertilization on the production and chemical composition of a grass crop grown under field conditions. At the same time, data were recorded on the seasonal trend in the dry-matter production and chemical composition of the grass. This enabled comparison of the results of these experiments with those of the indoor experiments and the field experiments in which light intensity was varied. Moreover it was possible to investigate, to what extend the seasonal trends depend on external conditions.

The lay out was designed to closely resemble the practical conditions, in which grassland is mown or grazed and fertilized. In 1962 the grass was harvested at an age of about 30 days, in 1963 at an age of 28 days.

4.3.1.1. Field experiments 1962

The experiments were set out in the spring of 1962 on a permanent pasture on a heavy river-clay loam and on a 2-years' ley on a light, drought sensitive, sandy soil poor in humus. *Lolium perenne* was the leading species in both pastures. The clover in the ley had been previously killed by spraying.

In periods of drought the water supply was kept at an adequate level by sprinkling. In spring 108 kg P_2O_5, 160 kg K_2O and 27.5 kg N/ha was supplied on the clay soil and on the sandy soil 85 kg P_2O_5, 80 kg K_2O and 45 kg N/ha. After each cut the plots were fertilized with 50 kg N/ha. One-month old grass was harvested every 10 days. There were three treatments. The harvest dates in these treatments were:

Treatment I	1/5	1/6	1/7	1/8	31/8	1/10
Treatment II	11/5	12/6	11/7	10/8	11/9	11/10
Treatment III	21/5	21/6	20/7	21/8	21/9	22/10

Each treatment had three replicates. The area of the plots was 5 × 1.50 m. On the date of cutting an area of 5 × 1.04 m was harvested. The following was determined:

dry-matter production and content;

chemical composition.

4.3.1.2. Field experiments 1963

The experiments carried out in 1963 were similar to those in 1962 and were also intended to investigate the effect of the soil type, nitrogen fertilization and moisture supply on growth during the season. In the autumn of 1962 experimental fields were set out on grasslands in the surroundings of Wageningen, situated on four soil types: a light, drought sensitive, sandy soil poor in humus, a moisture-retaining sandy soil, a heavy river-clay loam and a peat soil. On the light, sandy soil the experimental field was set out again on a ley, the other three soil types were under old permanent grassland. From this moment onwards the experimental fields were treated in the same way. On all soil types *Lolium perenne* was dominant and the clover was again killed by spraying. In spring a very liberal basic fertilization was applied: 120 kg P_2O_5/ha, 400 kg K_2O/ha in two equal doses and 150 kg MgO/ha.

The following treatments were applied on each experimental field:

two harvest dates (every 14 days 4-weeks' old grass was harvested);

two nitrogen levels; the plots with the high nitrogen level received 100 kg N/ha/cut until 28/6 and after this date 60 kg N/ha/cut; the plots with a low nitrogen level received 40 kg N/ha/cut until 14/6 and after this date 30 kg N/ha/cut;

two moisture levels (sprinkled in periods of water shortage and non-sprinkled.)

Each treatment had two replicates.

The harvest dates were:

Harvest date I	3/5	31/5	28/6	26/7	23/8	20/9	18/10
Harvest date II	17/5	14/6	12/7	9/8	6/9	4/10	1/11

The plots were 6×1.50 m². On the date of cutting 6.30 m² were harvested per plot. Periods of severe water shortage only occurred on the light, drought sensitive, sandy soil. The following was determined on the harvest dates:

dry-matter production and content;

chemical composition.

4.3.2. *Field experiment, spring 1964*

For a closer investigation into the influence of light intensity an experiment was set out in the spring of 1964, in which the light intensity was varied in the field.

The experiment was set out on heavy river-clay loam in a field of *Lolium perenne*, pasture type, var. Cebeco. The fertilization was 80 kg P_2O_5, 200 kg K_2O and 92 kg N/ha. The experiment lasted from 17 April to 13 May. The two light levels were obtained by placing cages over the grass, covered with cheese cloth or not. The calculated light levels in the experiment were:

L_1	88 cal cm^{-2}day^{-1}
L_2	282 cal cm^{-2}day^{-1}
(in the field	313 cal cm^{-2}day^{-1})

There were 8 replicates for each light level. The area per plot was 5×1.04 m². On the harvest date an area of 5×0.60 m² was cut. The following was determined:

dry-matter production and content;

chemical composition.

4.3.3. *Field experiment, autumn 1964*

This experiment was intended to investigate the influence of light intensity and nitrogen fertilization on the production and chemical composition of autumn grass. The experiment could be carried out after making arrangements, allowing the light intensity in the field to be increased as well. Thus it was possible to compare the chemical composition of the autumn grass and spring grass at about the same temperature and light intensity.

The experiment was set out on heavy river-clay loam on a first year ley of perennial ryegrass, pasture type, var. Pelo. The grass was sown under a cover crop of barley in May 1964. The experiment lasted from 17 September until 15 October. Besides treatments with a natural light intensity, there were those with a high and a low light intensity. The light intensities were obtained in the same way as in paragraph 4.2.1..

The average light levels during the experiment were:

L_1	48 cal $cm^{-2}day^{-1}$
L_2	240 cal $cm^{-2}day^{-1}$
L_3	547 cal $cm^{-2}day^{-1}$
(in the field	240 cal $cm^{-2}day^{-1}$)

The nitrogen applications were 25 and 125 kg N/ha. In each field there was a plot with an area of 3.50×1.50 m^2. On the harvest date 6 strips of 0.25×1.00 m^2 were cut per plot. The following was determined:
dry-matter production and content;
chemical composition.

4.3.4. *Twenty-five years' experiment 1938–1962*

The data of this experiment about which FRANKENA (1945) and BOSCH et al. (1963) have already reported, have been kindly put at our disposal by BOSCH.

This experiment is intended to study, in the course of many years, the influence of nitrogen fertilization and management on the soil and on the grass sward (BOSCH et al., 1963). The management on this experimental field varies from continually cutting to continually grazing (six treatments in all). The nitrogen applications were 80 and 200 kg N/ha/year, distributed over 4 to 5 cuts.

The experimental field is situated on a humous, sandy soil at Marum, about 150 km from Wageningen. The sward consists of many grass species, *Poa pratensis* being the dominant one.

The dry-matter production and chemical composition were determined of each cut in all treatments. However, the water-soluble-carbohydrate content and the nitrate content were not determined.

For our calculations the data were used on the dry-matter yield, length of the growing period, date of cutting and chemical composition of each harvest. In this the data on all treatments have been used, except those of the plots continually cut. The data on the latter treatments show a widely deviating production and botanical composition.

For each cut the average light intensity and temperature, during the four weeks preceding the harvest date, were calculated as indicated in paragraph 4.1..

All experiments with their treatments are summarized in appendix 1.

4.4. METHOD OF COLLECTING AND TREATING THE GRASS

The grass harvested always was about four weeks old. Grass of this age still is in full growth. The growing period of four weeks was selected because it corresponds well with grazing practices.

The following procedure was applied:

a. The object was to harvest the grass always within two hours after sunrise. For a comparison of the results it is advisable to standardize the harvest times. It is well known that the water-soluble-carbohydrate content increases under influence of the light during the day (cf. CURTIS, 1944). To prevent this light effect on the day of cutting the above-mentioned moment was taken. The grass was cut with an Agria-motor scythe in the experiments mentioned in the paragraphs 4.3.1., 4.3.2 and 4.3.4.; in the other experiments it was cut with a knife or shears. The cutting height of the motor scythe was about 5 cm. A cutting height of about 4 cm was realized with the knife or shears.

b. After cutting, the grass was immediately transported to the laboratory, where it was weighed and sampled upon arrival, which took maximally three hours.

c. The samples were dried in ventilated driers at a temperature of 70°C for 24 hours. After this treatment they were weighed again and ground.

d. The sand containing dry-matter content and yield were calculated from the data obtained. The water-soluble-carbohydrate content in the samples was usually determined at the author's laboratory. The other contents in the samples were determined at the Laboratory for Soil and Crop Testing at Oosterbeek. The samples of the experiments, mentioned in the paragraphs 4.2.2. and 4.3.2., were completely analysed by the Chemical Department of the Institute for Biological and Chemical Research on Field Crops and Herbage at Wageningen; those of the experiments, mentioned in the paragraphs 4.3.3. and 4.3.4. at the Laboratory at Oosterbeek.

e. The following was analysed in the ground samples:
dry-matter content, dried at 105°C;
nitrate content, by the xylenol-method;
crude-protein content, nitrogen content by the KJELDAHL-method × 6.25. In this method about 40% of the nitrate is lost. Because the nitrate content may be extremely high sometimes, the nitrate-free crude-protein content (=KJELDAHL N - 0.6 NO_3-N) × 6.25% is mostly applied in this publication;
ash content, dry matter ashed at 600°C;
sand content, ash insoluble in hydrochloric acid;
water-soluble-carbohydrate content, by Cu-reduction according to VAN DER PLANK (1936) revised by BOSMAN (1953);
crude-fibre content, substances insoluble in diluted H_2SO_4 and NaOH;
rest content, calculated from % r = 100 - % NO_3 - % cp - % ash - % wsc - % cf.

5. RESULTS AND DISCUSSION OF INDOOR EXPERIMENTS

For the time being, it is advisable to distinguish between the results of the indoor experiments and the field experiments. Therefore only the indoor experiments are treated in this chapter. In paragraph 5.1. the influence of climatical factors is first demonstrated with the results of one of the indoor experiments in 1963. Then, the indoor experiment of 1964 is dealt with in paragraph 5.2.; viz. afterwards it will be shown, that the nitrogen supply is important in explaining the reactions of the plant. In paragraph 5.3. all indoor experiments are compared, after which a general discussion follows in paragraph 5.4..

5.1. Indoor experiment 1963-III

To describe the above-mentioned effects the results of experiment 1963-III were selected, because the effects are most clear in this experiment, although they are evident in the other experiments as well. The results of this experiment are mentioned in figure 5 and in appendix 2 giving the results of all five indoor experiments 1963. First the moist treatments are discussed and subsequently the specific interactions with water shortage.

5.1.1. *Influence of light intensity*

Figure 5a shows that the fresh weight is relatively little influenced by the light intensity; the dry-matter content, however, is considerably raised by the increasing light intensity, as is the dry-matter yield.

With the high light intensity the nitrate content is very low and very high with the low light intensity. The crude-protein and ash contents, like the crude-fibre content, are low with the high light intensity. The water-soluble-carbohydrate content increases sharply with the light intensity; the rest content is also somewhat enhanced.

With a higher light intensity the nitrogen and ash quantity in the grass harvested are decreased, despite the higher dry-matter production, which is explained by nitrogen and ash also being stored in the roots and stubbles (cf. paragraph 3.2.2.). High light intensity has greatly stimulated the stubble and root

Tabel 14. Stubble and root weights of grass from the moist treatments in g dm pot^{-1} on 14/VI (data on 1 pot per treatment, averaged over the 3 temperatures of indoor experiment 1963 – III)

Light level	Stubble weight	Root weight
L_1	2.0	6.2
L_2	4.0	12.5
L_3	5.9	14.9

production; this is shown in table 14. The main cause of this high stubble production is the increased tiller formation in high light intensity.

If the crude-protein and ash contents in root and stubble are put at 10% (SULLIVAN et al., 1943) the uptake of nitrogen and minerals of the whole plant is almost independent of the light intensity. This may indicate that the nitrogen and minerals are depleted (stage t_3 in fig. 3), despite the liberal application.

5.1.2. *Influence of temperature*

Figure 5a shows that the fresh weight of the grass is increased at a higher temperature. The dry-matter content is also somewhat stimulated, thus the dry-matter yield increases with the temperature in this experiment.

The nitrate content is not influenced by the temperature. The crude-protein and ash contents, like the water-soluble-carbohydrate content, are low at the high temperature. The crude-fibre content is greatly and the rest content is somewhat increased with the temperature.

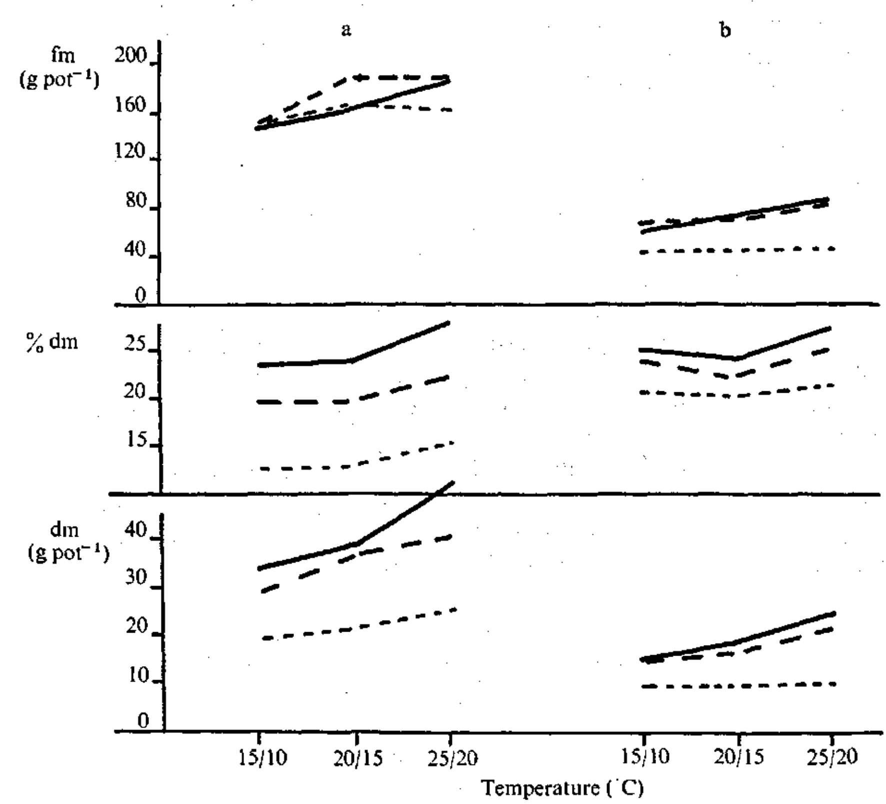
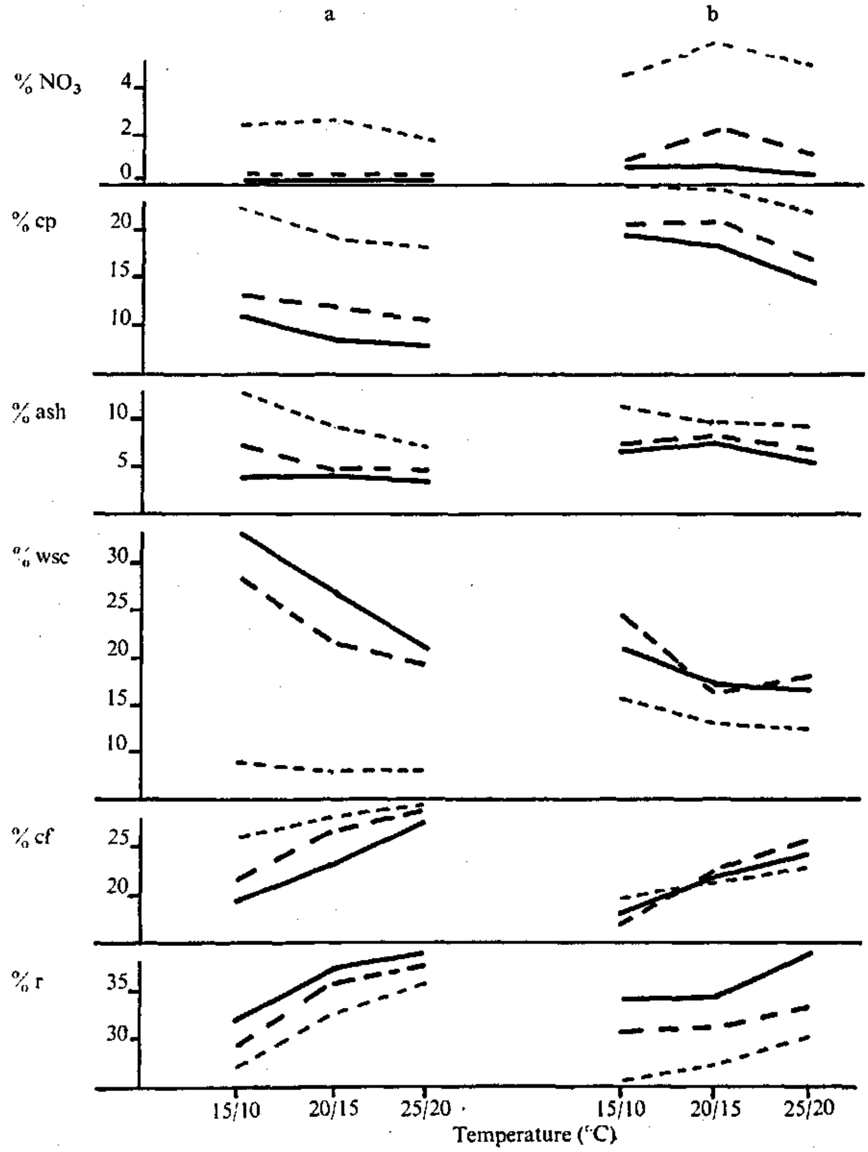


FIG. 5 Production and chemical composition of the grass of the 18 treatments from the indoor experiment 1963-III (Fig. 5a moist treatments, fig. 5b dry treatments; - - - - - low, — — — medium, ——— high light intensity)

FIG. 5 continued



At a high temperature the quantity of nitrogen and minerals in the grass cut was somewhat higher than at a low temperature. The stubble and root formation is hardly influenced by temperature, as is shown by table 15.

This indicates that, if the crude-protein and ash contents in stubble and root are put at 10%, the temperature has hardly affected the nitrogen and mineral uptake of the whole plant.

TABLE 15. Stubble and root weights of grass from the moist treatments in g dm pot^{-1} on 14/VI (data on 1 pot per treatment, averaged over the 3 light intensities of indoor experiment 1963–III)

Temperature	Stubble weight	Root weight
t_1	3.8	12.1
t_2	3.5	10.9
t_3	4.6	10.5

5.1.3. *Influence of water supply*

The data on the grass of the moist treatments are mentioned in figure 5a, those on the dry treatments in figure 5b.

These figures show that the fresh weight of the grass on the dry treatments is considerably lower than that of the moist ones. The dry-matter content is higher and the dry-matter yield lower on the dry treatments.

With this lower dry-matter yield the nitrate, crude-protein and ash contents are increased, while the water-soluble-carbohydrate, crude-fibre and rest contents are decreased. With water shortage the quantity of nitrogen and ash in the grass harvested is somewhat decreased; the stubble and root weights are also slightly lower (table 16).

These results indicate that the nitrogen and mineral uptake is retarded on the dry treatments.

TABEL 16. Stubble and root weights of grass in g dm pot^{-1} on 14/VI (average data on the moist and dry treatments of the indoor experiment 1963–III)

Water level	Stubble weight	Root weight
Moist	4.0	11.2
Dry	3.4	7.3

5.1.4. *Interaction of light intensity and water supply*

With some of the quantities measured, an interaction was found between light intensity and water supply. For instance, light intensity does not affect the fresh weight of the grass from the moist treatments, but has a positive effect on that from the dry treatments. The light intensity also affects the dry-matter content; on the moist treatments this effect is more evident than on the dry ones. Because of this the interaction on the dry-matter yield can no longer be observed.

Furthermore, the water-soluble-carbohydrate content in the grass is decreased by drought in the high light intensity, whereas it is increased in the low light intensity. This is reflected in table 17.

TABEL 17. Water-soluble-carbohydrate contents of grass with different light intensities and water supplies (data averaged over the 3 temperatures of the indoor experiment 1963–III)

Water level	Light intensity		
	L_1	L_2	L_3
Moist	8.3	22.8	27.0
Dry	13.4	19.3	18.3

5.2. INDOOR EXPERIMENT 1964

In paragraph 4.2.2. it was already mentioned that the light intensity and the nitrogen plus mineral supply were varied in this experiment.

The light intensities, nitrogen and mineral applications in this experiment were widely varied, resulting in extreme values being found in the chemical composition. The results are mentioned in figure 6 and in appendix 3.

5.2.1. *Influence of light intensity*

Figures 6 shows that the fresh weight, averaged over the fertilization levels, increases with the light intensity. The dry-matter content is also stimulated and as a result the dry-matter yield increases with the light intensity.

In high light intensity the nitrate, crude-protein and ash contents are low again, similar to the indoor experiment 1963-III. Under these conditions the water-soluble-carbohydrate content is again high, whereas the crude-fibre content is low. The rest content is hardly influenced in this experiment.

In the high light intensity the quantity of nitrogen and minerals in the grass is the highest on an average. It is striking that the effect of the light intensity between 90 and 260 cal $cm^{-2}day^{-1}$ is much greater than that between 260 and 540 cal $cm^{-2}day^{-1}$.

The stubble and root production is appreciably stimulated by the light intensity, as may be seen from table 18.

TABLE 18. Stubble and root weights of grass in g dm pot^{-1} on 22/V in various light intensities (data on 3 pots per treatment, averaged over the fertilization levels of the indoor experiment 1964)

Light intensity	Stubble weight	Root weight
L_1	3.2	7.2
L_2	6.1	11.0
L_3	8.0	14.1

Assuming a crude-protein and ash content of 10% in stubble and root, the uptake of nitrogen and minerals by the plant will show a relatively sharp increase with the light intensity.

5.2.2. *Influence of fertilization*

The fresh weights are considerably stimulated by fertilization, whereas the dry-matter content is reduced. The dry-matter yield as a result is also stimulated.

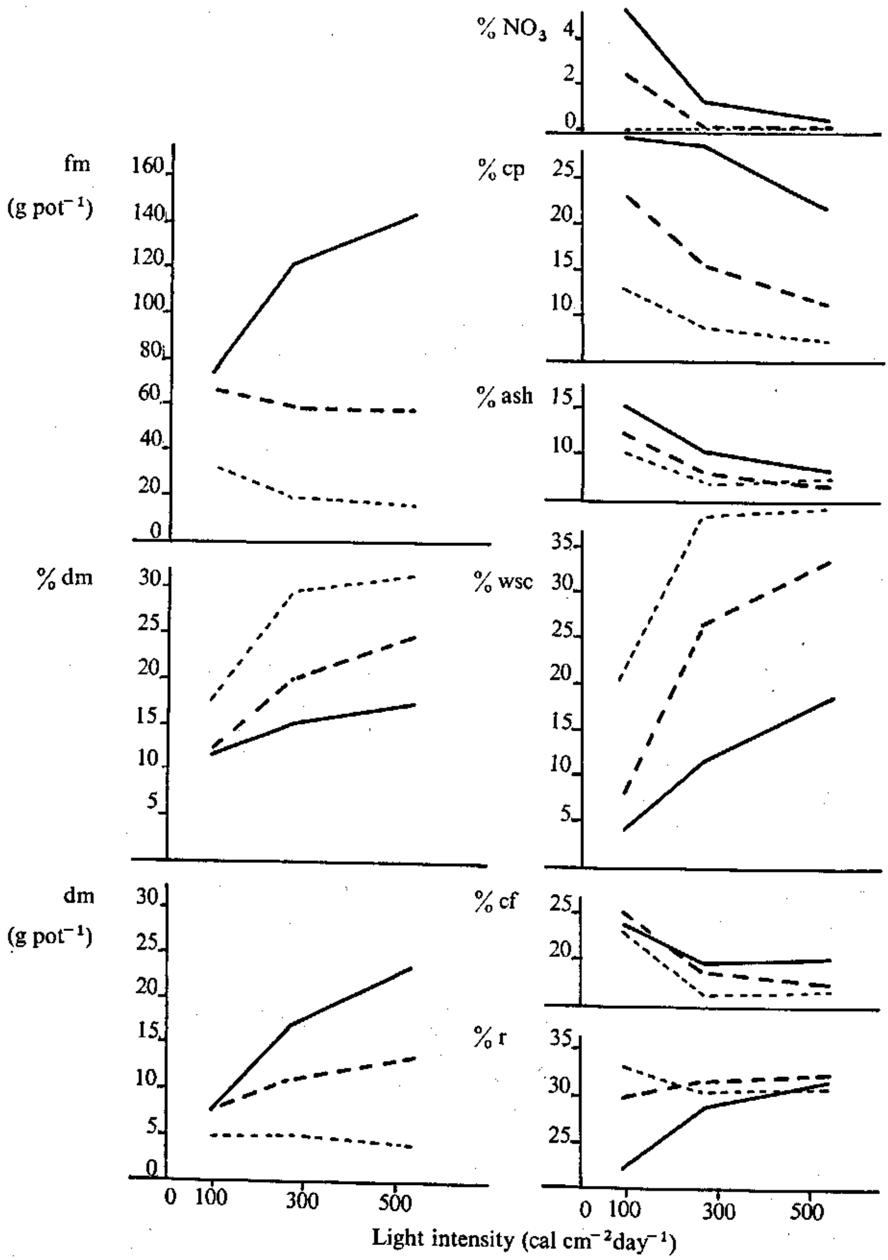


FIG. 6 Production and chemical composition of the grass of the 9 treatments from the indoor experiment 1964; (- - - - - low, – – – medium, ——— high fertilization)

The nitrate, crude-protein as well as the ash contents are increased under these conditions. The water-soluble-carbohydrate content is appreciably reduced. The crude-fibre content is higher, whereas the rest content is not much influenced.

The quantity of nitrogen and ash in the grass is also raised by fertilizer application. The stubble and root production is hardly affected by the fertilization (cf. table 19).

TABLE 19. Stubble and root weights of grass in g dm pot^{-1} on 22/V at different fertilization levels (data on 3 pots per treatment, averaged over the light levels of the indoor experiment 1964)

Fertilization	Stubble weight	Root weight
N_1	4.8	10.7
N_2	5.9	11.8
N_3	6.6	10.1

Because of this small fertilizer effect on the stubble and root production, its influence on the total uptake of nitrogen and minerals is also positive.

The results of this experiment show that the light intensity and fertilization stimulate the fresh-matter as well as the dry-matter yield, but they counteract each other in the chemical composition.

5.2.3. *Interaction of light intensity and fertilization*

Figure 6 shows that this interaction is almost absent in the chemical composition. Only with the low fertilization the nitrate content was not affected, because the content was already very low in the low light intensity. The rest content only was reduced in the low light intensity by the fertilization.

However, this interaction is distinctly evident in the yields. At the low fertilization level the light intensity does not affect the dry-matter yield, but at the high level the influence is considerable.

Because the plants contain less water in a high light intensity the light even has a negative effect on the fresh-matter yield of the treatments with a low or moderate fertilization, whereas the influence is clearly positive in the treatments with a high fertilization.

Since the interaction does occur with the dry-matter yield, but not with the crude-protein and ash content, it is also observed in the quantity of nitrogen in the grass harvested. With this the light effect is negative with a low fertilization level and positive with a high application. It is striking, that with the high application the nitrogen quantity increases with the light intensity up to 260 cal $cm^{-2}day^{-1}$, but this increase does not continue above this level.

5.3. Comparison of the results of the various indoor experiments

The results of the indoor experiments 1963-II–VI suggest the way in which grass, growing in the different parts of the season, reacts on light, temperature and water supply. At the same time the effect of shooting of the grass may be examined. The results of the indoor experiment 1964 may be applied in explaining certain effects of the nitrogen supply.

For this the results of the experiments 1963 have been:

a. averaged over the three temperatures and plotted against the light intensity (moist treatments only);
b. averaged over the three light intensities and plotted against the 24-hours' temperature (moist treatments only);
c. averaged over the two moisture levels and plotted against the water supply.

The results are shown in figure 7a, b and c.

The results of the moderately fertilized treatments of the indoor experiment 1964 have been included in the figure as well.

5.3.1. *Level differences between the experiments.*

First of all it is noticed in figure 7a, that there are considerable differences in the dry-matter production between the experiments. To obtain reliable results these differences are studied at a light intensity of about 300 cal $cm^{-2}day^{-1}$.

The dry-matter production in the late summer and autumn experiments (experiments 1963-IV, -V and -VI) with grass in a ráther vegetative stage is almost the same. That of the experiments in spring with purely vegetative grass (indoor experiment 1963-II and indoor experiment 1964) is greatly retarded, whereas that of the shooting grass in the indoor experiment 1963-III is considerably higher.

Before drawing any conclusions the experimental conditions are to be examined more closely.

a. All pots of the indoor experiment 1963 were planted in November 1962. This means that the pre-experimental period of the grass was different for each experiment. This leads to differences in the tiller number as well as in the stubble and root weights as shown in table 20.

TABLE 20. Condition of the pots at the beginning of the different parts of the indoor experiments

Experiment	Number of tillers per pot	Stubble weight (g dm pot^{-1})	Root weight (g dm pot^{-1})
1963– II	186	2.4	6.6
1963–III	222	2.9	4.5
1963–IV	305	3.8	9.2
1963– V	354	6.5	12.1
1963–VI	351	8.9	14.4
1964–N_2	360	3.8	7.1

This table may suggest that the low yield of experiment 1963-II, compared to the experiments 1963-IV, -V and -VI, may have been the result of the small number of tillers and the low stubble and root weights. This does not hold, however, for the also purely vegetative grass of indoor experiment 1964. The high dry-matter yield in experiment 1963-III can neither be explained from the tiller number nor from the stubble and root weights.

b. Experiment 1963-II was started with an almost bare stubble, without green leaves, the other experiments were started with a somewhat green stubble.

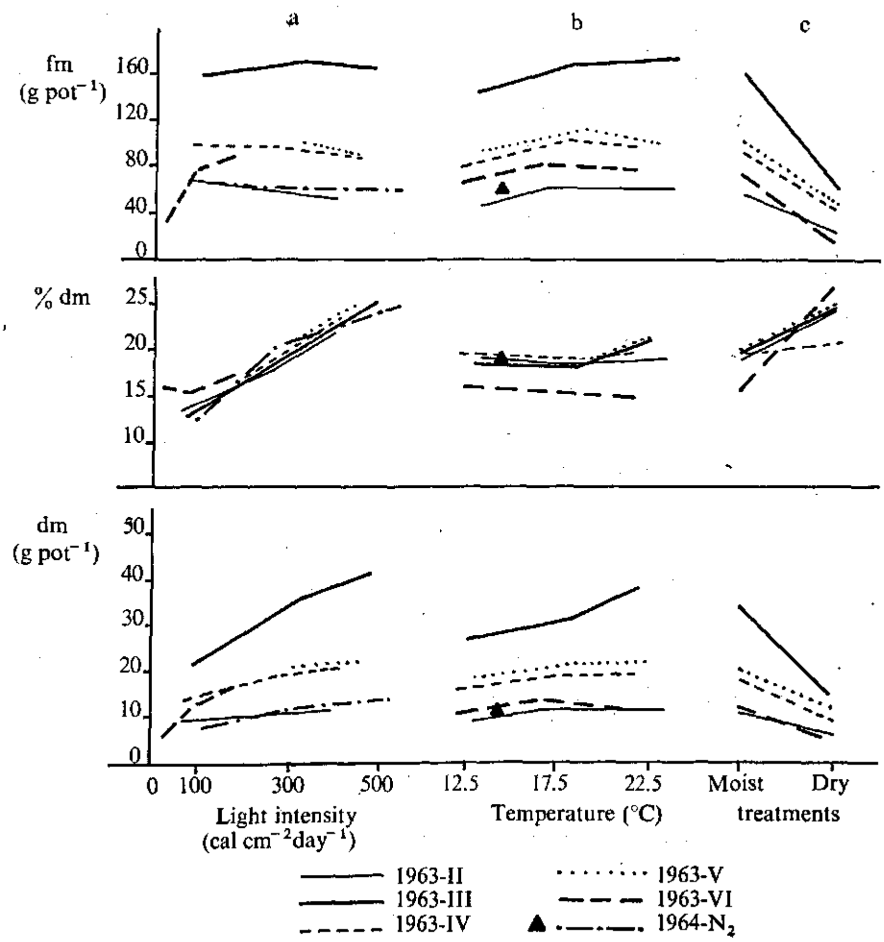
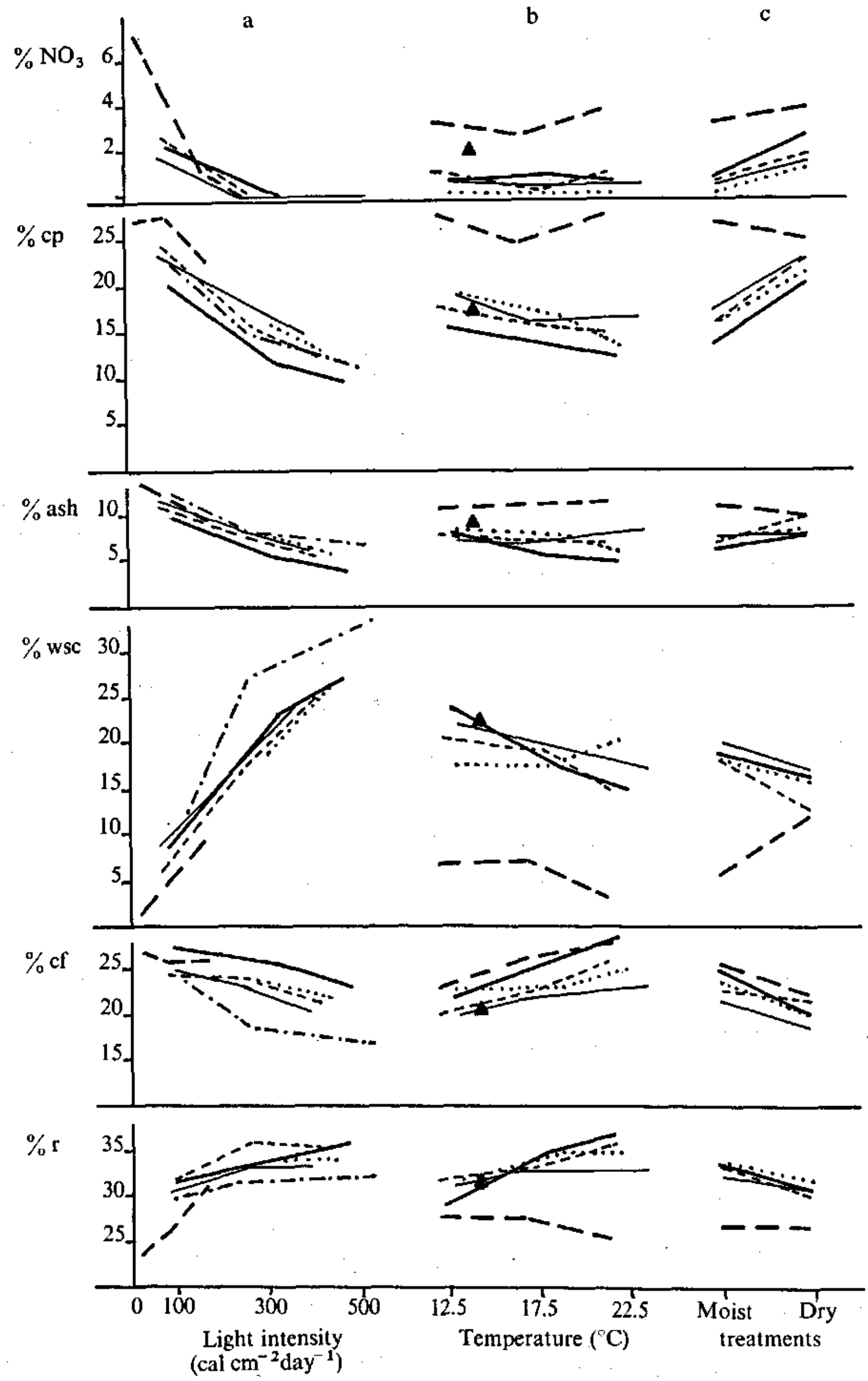


FIG. 7 Production and chemical composition of the grass from all indoor experiments

a. averaged over the three temperatures and plotted against the light intensity (moist treatments only)
b. averaged over the three light intensities and plotted against the temperature (moist treatments only)
c. averaged over the two moisture levels and plotted against the water supply

FIG. 7 continued



The effect of this, however, can neither explain the difference.

c. The mineral supply in the six experiments was different. DE WIT et al. (1963) state that the nitrogen and potassium supply are not limiting factors, when per gram of dry matter about 3 meq.N and about 0.7 meq. K are available. These figures are mentioned for the different experiments in table 21.

TABLE 21. Nitrogen and potassium application in meq. per gram of dry matter (averages of the moist treatments of the indoor experiments)

Experiment	meq. N/g dm	meq. K/g dm
1963– II	3.2	0.85
1963–III	1.4	0.24
1963–IV	2.6	0.5
1963– V	3.2	0.85
1963–VI	4.0	0.7
1964–N_2	3.0	0.8

In experiment 1963-III the nitrogen and potassium application remained far below the mentioned standards. The dry-matter production, nevertheless, is the highest in this experiment. This means that in this case the low nitrogen and potassium fertilization did not considerably retard the dry-matter production.

The only explanation remaining is that the grass in experiment 1963-III had a much more rapid rate of growth from itself than in the other experiments; the shooting of the grass should probably be seen therefore as a positive growth factor.

The dry-matter content in the grass has been almost the same in all experiments. Only in experiment 1963-VI it is about 2% higher. The dry-matter content being the same in all the other experiments is, however, very striking. It has been found in the indoor experiment 1964, that differences in the fertilization do affect the dry-matter content.

Where the differences in the dry-matter yield between the experiments are considerable, it might be expected that the chemical composition of the grass in the various experiments should also vary widely. Nevertheless, these differences were relatively small.

Figure 7a shows that the nitrate content in the grass is usually low.

The crude-protein content in the grass is lowest in experiment 1963-III. In the experiments 1963-II, -IV, -V and the indoor experiment 1964 it is about 2–4% and in experiment 1963-VI about 7% higher. The reason for the higher content in the latter experiment is presumably the somewhat higher nitrogen fertilization (table 21).

The ash content in the grass is almost the same in all the experiments. The differences in mineral application (table 21) did not lead to differences in the ash content.

The water-soluble-carbohydrate contents in the grass do not vary considerably in the different experiments. In experiment 1963-II and -III the contents are $1\frac{1}{2}$–2% higher than in experiment 1963-IV and -V. The equal water-soluble-carbohydrate contents in the experiments 1963-II and -III may indicate that shooting does not greatly affect this content. In experiment 1963-VI the content is 3.5% lower, probably resulting from a somewhat more liberal nitrogen application. The higher content in the indoor experiment 1964 may have been caused by the low temperature (see paragraph 5.3.3.).

Excluding the indoor experiment 1964, the crude-fibre content is lowest in the grass of experiment 1963-II and highest in experiment 1963-III. The difference is about 4%. The crude-fibre contents in the other experiments are intermediate. Maybe the higher content in experiment 1963-III is caused by the rather great number of inflorescences. The low content in the grass of the indoor experiment 1964 was brought about by the low temperature in this experiment (see paragraph 5.3.3.).

The rest content in the grass is almost the same in the various experiments; it is about 3.5% lower in experiment 1963-VI only. The slightly lower content in the indoor experiment 1964 is again associated to the low temperature.

5.3.2. *Influence of light intensity*

Figure 7a shows that the fresh weight is hardly influenced by the light intensity, the dry weight, however, is all the more so. The effect of the light intensity on the dry-matter production is greater with a higher production level. This probably is associated with the light interception by a larger area. For in these experiments in which the grass is grown in pots, the shoots with leaves of plants with a rapid growth rate may deflect horizontally sooner to intercept more light than those of plants with a slower growth rate.

The figure shows that the curves do not run through the origin. This may be because only the overground yield has been plotted in the figures. Since the plant adjusts the dry-matter distribution to the light intensity, a greater share of the assimilates remains overground in a low light intensity. If the total dry-matter production would be plotted against the light intensity, also taking into account the photosynthesis curve in figure 1, the curves might run through the origin.

In experiment 1963-VI the light effect deviates from that in the other experiments. It probably are the small reserves in the stubbles and roots at the beginning of the experiment, which cause difficulties for the plant to adjust itself to the very low light intensity.

In all experiments the influence of the light intensity on the dry-matter content is about the same. The dry-matter content increases with about 2.7% per 100 cal $cm^{-2}day^{-1}$. The effect is somewhat smaller in experiment 1963-VI only. This may be the result of the more liberal nitrogen supply. The indoor experiment 1964 shows that the light effect is reduced with more liberal applications. The light effect in the indoor experiments 1963-II–V and experiment 1964-N_2 being the same, may possibly indicate, that nitrogen depletion was achieved in each case (stage t_3 in figure 3).

Figure 7a proves that the light intensity has about the same effect on the chemical composition in all experiments. In the higher light intensity the nitrate, crude-protein, ash and crude-fibre contents are always reduced and the water-soluble-carbohydrate content is stimulated. The rest content is also increased, except for the treatment with the low fertilizer application in the indoor experiment 1964.

This light effect on the constituents of the dry matter treated in all experiments, is mentioned in table 22.

TABLE 22. Effect of 100 cal $cm^{-2}day^{-1}$ in the range of 100–500 cal $cm^{-2}day^{-1}$ on the chemica composition of the grass in the different parts of the indoor experiments

Experiment	% NO_3	% cp	% ash	% wsc	% cf	% r
1963– II	-0.35	-2.6	-1.5	+5.0	-1.3	+0.8
1963–III	-0.50	-2.7	-1.6	+4.7	-1.0	+1.2
1963–IV	-0.58	-3.1	-1.5	+5.3	-0.9	+0.9
1963– V[1])	-0.02	-2.4	-1.1	+4.4	-0.7	+0.1
1963–VI[2])	-3.8	-3.5	-3.2	+5.5	-0.8	+5.5
1964–N_1	-0.0	-1.4	-0.7	+4.1	-1.6	- 0.6
1964–N_2	-0.5	-2.9	-1.3	+5.9	-1.9	+0.4
1964–N_3	-1.2	-1.7	-1.7	+3.2	-0.9	+2.0
Average effect[3])	-0.45	-2.4	-1.3	+4.7	-1.2	+0.7
Average content[3])	1.36	18.5	8.6	16.4	23.5	31.6

[1]) calculated in the range of 300–500 cal $cm^{-2}day^{-1}$
[2]) calculated in the range of 50–150 cal $cm^{-2}day^{-1}$
[3]) without the indoor experiment 1963–VI

Figure 7a and table 22 show that the results in experiment 1963-VI deviate somewhat from those in the other experiments. This may have been the effect of the low light intensity during the experimental period. Therefore this experiment was left out of consideration in calculating the averages in table 22. The average light effects in table 22 show that the water-soluble-carbohydrate content is most affected by light intensity. The crude-protein content is also appreciably influenced, but the nitrate, ash, crude-fibre and rest contents react to a less extent on the light intensity.

Table 22 suggests: 1. that the reaction of the chemical composition of the grass, growing in the different times of the season, to the light intensity is almost the same (indoor experiments 1963) and 2. that the fertilizer application does not seriously affect the size of the light effect.

5.3.3. *Influence of temperature*

In calculating the effect of the temperature it is not advisable to proceed from the data averaged over the light levels. To obtain comparable figures the dry-matter yields and chemical composition were calculated by interpolation, in some cases by extrapolation, in 100, 300 and 500 cal $cm^{-2}day^{-1}$. The average result has been plotted in figure 7b.

In all experiments the fresh-matter yield increases from 15/10°C to 20/15°C, but there is little or no rise from 20/15°C to 25/20°C. The dry weight reacts in in the same way, experiment 1963-III excepted, where it is advanced to an increasing extent from 20/15°C to 25/20°C. Maybe that at the high temperature the shooting of the grass in experiment 1963-III led to a more favourable spatial arrangement of stems and leaves for utilizing the light.

The dry-matter production at 20/15°C and 25/20°C being almost the same in the other experiment, indicates that the optimum temperature is in this range. This agrees with the observations of ALBERDA (1957), DEL POZO (1963) and MITCHELL (1956) in their experiments with *Lolium perenne*.

The dry-matter content is hardly influenced by temperature. Only in the experiments 1963-III and -V there is a slight increase from 20/15°C to 25/20°C. MITCHELL (1956) indeed found a temperature effect in the range 7–35°C: the content was lowest at the optimum temperature. However, near the optimum temperature the differences in the dry-matter content were small. GROBBELAAR (1963) mentions similar results in maize. The temperature range in the present experiments probably was not wide enough and too near the optimum temperature to find a measurable temperature effect.

Temperature has a relatively great influence on the chemical composition of the grass. Figure 7b shows that the nitrate content is only slightly affected by the temperature. The crude-protein, ash and water-soluble-carbohydrate contents usually are reduced by a higher temperature. Probably the water-soluble-carbohydrate content in experiment 1963-V is unreliably high in the treatment 25/20°C in the normal light intensity. For this reason the temperature effect on this content deviates in that experiment. The crude-fibre content is continually and the rest content usually higher at a higher temperature.

The data of the indoor experiment 1964-N_2 have been included in figure 7b. It can be seen that these points correspond well with those of the indoor experiments 1963. The lower crude-fibre and rest content and the higher water-soluble-carbohydrate content in the grass of the indoor experiment 1964, in figure 7a, almost certainly are the result of the low temperature. In table 23 the before-mentioned temperature effects are reflected quantitatively.

It can be seen from this table that the water-soluble-carbohydrate and crude-fibre contents are most affected by temperature; whereas the crude-protein and rest contents are influenced to some extent and the nitrate and ash contents are hardly affected at all.

Analogue to what was said about the light intensity, it may be inferred from table 23 that the chemical composition of the grass, growing in the different times of the season, always reacts in about the same way to the temperature.

If the results of the experiments 1963-II–VI are treated together by a multiple regression technique, the following regression equations are found (table 24).

(R^2 is the quadrate of the correlation coefficient and also the estimation of the explained part of the original variation; s is the estimation of the standard de-

TABLE 23. Effect of 1 °C in the range between 15/10°C and 25/20°C on the chemical composition of grass in the different parts of the indoor experiments 1963

Experiment	% NO_3	% cp	% ash	% wsc	% cf	% r
1963– II	−0.02	−0.24	+0.12	−0.46	+0.36	+0.22
1963–III	−0.02	−0.32	−0.30	−0.90	+0.66	+0.80
1963–IV	0	−0.28	−0.10	−0.62	+0.64	+0.46
1963– V[1])	−0.02	−0.60	−0.22	+0.26	+0.22	+0.34
1963–VI[2])	+0.08	+0.10	+0.06	−0.44	+0.52	−0.28
Average effect	+0.00	−0.27	−0.09	−0.43	+0.48	+0.31
Average content	1.42	18.7	8.5	15.2	24.3	32.0

[1]) measured in 300 cal $cm^{-2}day^{-1}$

[2]) measured in 100 cal $cm^{-2}day^{-1}$, because the contents cannot be estimated sufficiently accurate in 300 and 500 cal $cm^{-2}day^{-1}$

TABLE 24. Regression equations of the dry-matter yield and some contents calculated from the experiments 1963–II–VI (x_1 = light intensity in cal $cm^{-2}day^{-1}10^{-2}$, x_2 = 24-hours' temperature in °C)

	Regression equation	R^2	s
dm (g pot^{-1})	$= 0.4 + 4.1\ x_1 + 0.45\ x_2$ (NS)	0.44	7.9
% cp	$= 32.0 - 3.57\ x_1 - 0.276\ x_2$	0.87	2.3
% ash	$= 11.7 - 1.58\ x_1 + 0.051\ x_2$ (NS)	0.51	2.4
% wsc	$= 9.4 + 5.54\ x_1 - 0.434\ x_2$	0.92	2.6
% cf	$= 18.4 - 0.96\ x_1 + 0.473\ x_2$	0.66	1.7
% r	$= 22.7 + 1.80\ x_1 + 0.292\ x_2$	0.60	2.6

viation, the standard of the reliability of the dependent variable, if calculated from the independent variables).

In comparing the tables 22, 23 and 24 it can be seen, that the regression coefficients in table 24 generally correspond fairly well with the average effects in the tables 22 and 23.

5.3.4. *Influence of water supply*

The average data can neither serve as a basis for calculating the influence of the water supply. Therefore the average dry-matter yield and the chemical composition of the moist as well as the dry treatments have been calculated in 300 cal $cm^{-2}day^{-1}$ and at 15/10°C, 20/15°C and 25/20°C. This was not applied in experiment 1963-VI, because the light intensity was too low for a reliable extrapolation. For this experiment the water effect is mentioned with 100 cal cm^{-2} day^{-1}.

The water shortage, brought about in the indoor experiments 1963, always retarded the dry-matter production and with it influenced the chemical composition (see figure 7c.) Under the dry conditions the dry-matter content is stimulated, like the nitrate, crude-protein and ash contents, whereas the water-soluble-carbohydrate, crude-fibre and rest contents are reduced. The reaction of the grass deviating on some points in experiment 1963-VI is probably caused by the low light intensity and the differences in nitrogen application between the moist and dry treatments.

5.3.5. *Interaction of light intensity and water supply*

In indoor experiment 1963-III and also in the other experiments it was found, that in a high light intensity the water-soluble-carbohydrate content in the grass of the moist treatments is higher than in that of the dry treatments. In a low light intensity it is the reverse. In paragraph 5.4.5. this is discussed.

5.4. DISCUSSION

5.4.1. *Dry-matter production*

As expected the dry-matter yield increases with the light intensity in the indoor experiments. This rise, however, is not found with extreme nitrogen and mineral deficiencies, see indoor experiment 1964 with the low fertilization. It was also found in this experiment, that the light effect is stimulated by a more liberal fertilization. This may indicate that the light effect on the dry-matter production in the indoor experiments 1963-II–V could have been higher, if the fertilizer application had been higher.

The indoor experiment 1964 showed that the fertilization effect in low light intensity is slight, but in high light intensity it is considerable. To turn the periods with high light intensity to advantage a much more liberal fertilizer treatment will have to be applied.

Only a small positive effect of the temperature on the dry-matter yield was found in the separate experiments; this effect even disappears when all experiments are treated together. The temperatures in these experiments probably were too near the optimum to find appreciable temperature effects on the dry-matter yield.

5.4.2. *Nitrate*

In paragraph 3.2.1. it has already been mentioned that nitrate is a nutrient for the plant. It is taken up from the root medium and used by the plant in the formation of different nitrogen-containing substances.

When the nitrate concentration in the root medium is high, much nitrate is taken up the and content in the plant will be high, cf. indoor experiment 1964.

The nitrate taken up is distributed over the whole plant. If the soil-nitrogen supply is limited, all is taken up. In this case the content in the plant may be about inversely proportional to the dry-matter yield. This may be found in the indoor experiments 1963 with a limited nitrogen supply under influence of the temperature.

If there is excess nitrate in the root medium the uptake may be limited by the temperature. The findings of ALBERDA (1965) show that the nitrate uptake of the plant and the nitrate content in the leaf increased with the temperature. The nitrate uptake was somewhat reduced at 30°C only. This high nitrate content at a rather high temperature was also found in the indoor experiment 1963-VI in the low light intensity.

It has already been mentioned in paragraph 3.2.1., that the nitrate is reduced before the plant can utilize it. Nitrate reduction especially takes place under influence of the light. Therefore the nitrate content is mostly high in a low light intensity and always low in a high one, cf. indoor experiments 1963, even with a liberal nitrogen fertilization (indoor experiment 1964).

The nitrate content in the indoor experiments 1963 was always increased by water shortage. This may have been caused by a retarded nitrate reduction, but also by the hampered dry-matter production, resulting in more nitrate being available on the harvest date.

In a number of publications, ALBERDA (1962; 1965), VAN BURG (1962; 1965) the nitrate content in the grass is used as a standard for the nitrogen supply to the crop. With concentrations over 0.6% NO_3 an additional fertilization with nitrogen is supposed not to affect the dry-matter production. Because of the light effect on the nitrate reduction, it is likely, that this value will be lower in a higher light intensity: VAN BURG (1962) already suggested this, because in the low light intensities in autumn he obtained the maximal production with nitrate contents higher than 0.6%.

5.4.3. *Crude protein*

In paragraph 3.2.2. it was shown that the crude-protein content may be approximated by:

$$\% \text{ cp} = \frac{\text{N-uptake} \times 625}{\text{dry-matter yield}}$$

Figure 3 represents the trend in this nitrogen uptake and dry-matter yield of the whole plant. The results obtained by the indoor experiments correspond well. This is shown in figure 8, reflecting the total dry-matter yield and nitrogen uptake of the grass of all treatments in the indoor experiments 1963-II and -III. The points, however, are somewhat scattered which may have been mainly caused by determination errors of the yields and of the nitogen contents in the grass, stubbles and roots.

The vertical line in figure 3a with complete nitrogen depletion is only found in the grass of the moist treatments in the indoor experiment 1963-III. In all the other experiments more or less sloping lines indicate the correlation between the dry-matter production and nitrogen uptake. In the experiments various transitions were obtained from nitrogen excess to nitrogen depletion.

In a high light intensity the dry-matter yield of the grass may be high. This indicates that with a low nitrogen fertilization the light effect on the nitrogen uptake is low, but it will be high with a liberal application.

In these experiments the temperature effect was small on the relationship between the dry-matter production and nitrogen uptake. Water shortage has reduc-

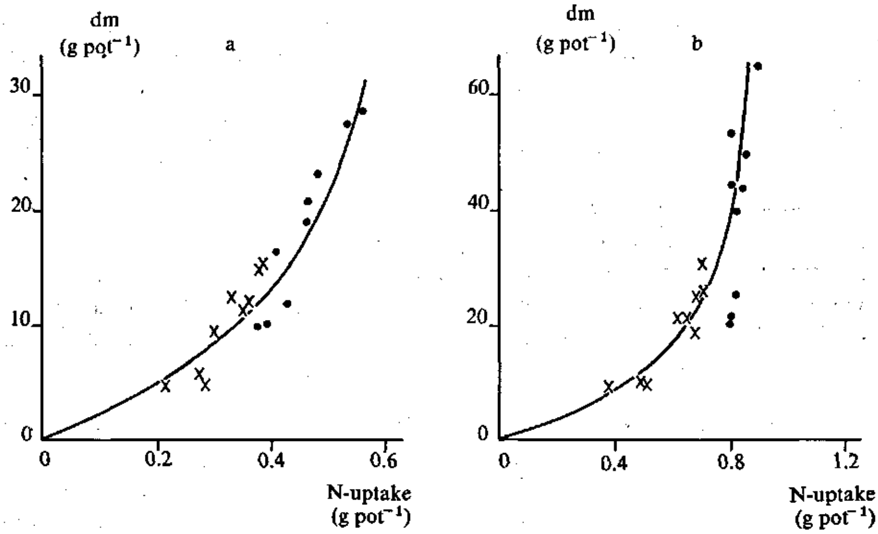


FIG. 8 Dry-matter production and nitrogen uptake of the whole grass plant of all treatments from the indoor experiment 1963-II (fig. 8a) and 1963-III (fig. 8b)
(● moist treatments; × dry treatments)

ced the dry-matter yield and the nitrogen uptake; the former, however, to a larger extent than the latter.

Considering the correlation between the dry-matter yield and the quantity of nitrogen in the grass harvested, it is found that this relationship has changed, influenced by the stubble and root production and the nitrogen stored in these. Thus the dry-matter yield is usually higher and the quantity of nitrogen in the grass lower by increasing the light intensity, as has been indicated in paragraph 3.2.2.. The influence of the light intensity on the crude-protein content is therefore considerable.

The nitrogen quantity in the grass of experiment 1963-VI and of the indoor experiment 1964-N_3 increases with the light intensity because of the nitrogen excess. Yet here too, a higher light intensity will decrease the crude-protein content considerably, whereas figure 3 indicates that a light effect on this content under these conditions of nitrogen excess is supposed to be absent.

The dry-matter yield and nitrogen quantity in the grass are both somewhat stimulated by the temperature. Therefore the crude-protein content will only change slightly with the temperature.

With water shortage the points of the dry-matter yield and nitrogen quantity are on the sloping part of the line in figure 8, consequently in the range of nitrogen excess. The points are rather well around the curve. The variation in the crude-protein content is therefore smaller under dry conditions than under moist conditions. All the same, light and temperature show a similar effect under dry conditions to under moist conditions.

5.4.4. *Ash*

Like the crude-protein content the ash content in the plant can be approached by:

$$\%\ \text{ash} = \frac{\text{ash quantity} \times 100}{\text{dry-matter yield}}$$

The indoor experiment 1964 shows that the concentration of ash in the grass is higher with an increasing mineral supply. The mineral uptake of the grass is more stimulated by the fertilization than the dry-matter production, and in four weeks' old grass the ash content will therefore increase with the fertilization.

In the indoor experiments 1963 the uptake of minerals by the whole plant is little influenced by the light intensity. In the grass harvested it usually decreases somewhat with increasing light intensity. Since the dry-matter yield is raised by increasing light intensity, the ash content will rather sharply decrease.

A rise in temperature, on the other hand, has a positive effect on the ash quantity in the grass. The dry-matter production is usually affected to the same extent, resulting in the ash content not being influenced by the temperature in a number of cases. Only in experiment 1963-III, in which shooting grass was used and in which the temperature effect on the dry-matter yield was considerable, a negative effect of a temperature rise was found on the ash content.

Water shortage, as brought about in the indoor experiments 1963, retards the dry-matter production more than the mineral uptake; this leads to a somewhat higher ash content under dry conditions.

In general, the ash content reacted in the same way to the external conditions as the crude-protein content. Only their reaction to temperature was different. Probably, this response would have been the same with a more liberal nitrogen supply.

5.4.5. *Water-soluble carbohydrate*

In paragraph 3.2.4. it was stated that the water-soluble carbohydrates may be seen as intermediate products, the content being dependent on their supply and use. The carbohydrate supply is determined by the light intensity and the use by the transport to stubbles and roots, respiration, formation of tissues and protein synthesis. The tissue formation and protein synthesis are especially stimulated by the nitrogen fertilization.

A reasonable consequence is that the nitrate or crude-protein content and the water-soluble-carbohydrate content are often found to be correlated. ALBERDA (1965) mentions negative correlations of the nitrate content with the water-soluble-carbohydrate content and DE ROO et al. (1963) find a negative correlation of the crude-protein content with the water-soluble-carbohydrate content. The above-mentioned relationships have also been investigated in the present experiments. Figure 9 demonstrates the correlation of the nitrate content with the water-soluble-carbohydrate content in some of the experiments.

As the figure shows, the line reflecting the relationship is a hyperbole. With high nitrate contents the carbohydrate content is low, initially it will increase

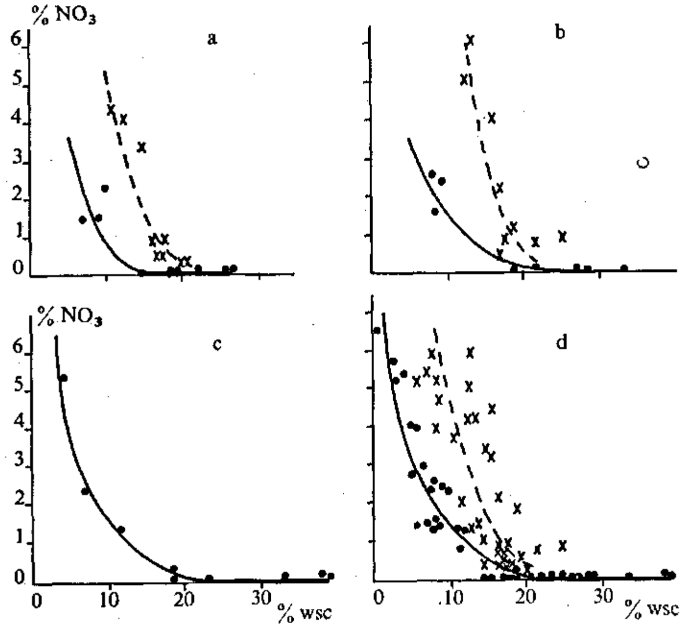


FIG. 9 Relationship between the water-soluble-carbohydrate content and the nitrate content of the grass from the moist (—— •) and dry (- - - - - ×) treatments
a. Indoor exp. 1963-II   c. Indoor exp. 1964
b. Indoor exp. 1963-III   d. All indoor exp. 1963, 1964

slightly with a decreasing nitrate content. However, the carbohydrate content will rise more considerably with a reducing nitrate content and finally with very low nitrate contents it will achieve very high values. It is striking that the hyperbole for the moist treatments is the same in all experiments.

Influenced by the light intensity the points move along the hyperbole to higher carbohydrate and lower nitrate contents; influenced by the nitrogen application the reverse will take place. The temperature neither affects the place of the hyperbole relative to the axes, unlike the water supply which does show an influence: under dry conditions the hyperbole has moved to higher carbohydrate contents.

The hyperboles drawn in figure 9 for the grass of the moist treatments only with the low carbohydrate content correspond in place with those of the grass in the experiments of ALBERDA with nitrogen excess. They closely resemble those of the grass in his short experiment at 25°C and nitrogen depletion. This may not be considered as evidence, however, of the hyperbole in the present experiments being brought about by nitrogen depletion, because in that case the points from the indoor experiment 1964 could not have been in one line.

The difference in the hyperboles of these experiments and ALBERDA's is probably caused by the light intensity. In the present experiments the highest light intensities were 2.5 to 3 times higher than those in ALBERDA's experiments. This higher light intensity led to an increased nitrate reduction and therefore lower nitrate contents were found, which may have caused the resemblance with eht above-mentioned depletion curve.

In the present experiments only a slight influence of the temperature on the place of the hyperbole was found, whereas ALBERDA found a distinct temperature effect. This may be associated to the experimental technique: in the present experiments with a growing period of four weeks the temperature did not affect the nitrogen uptake and the nitrate content, but this did occur in ALBERDA'S two weeks lasting experiments.

The hyperbole is moved to higher carbohydrate contents by water shortage. This is caused by the depressed growth rate in dry conditions, resulting in a smaller use of sugar.

However, it is demonstrated in the paragraphs 5.1.4. and 5.3.5., that in the higher light intensities the carbohydrate content was decreased by drought. In these conditions with the same nitrogen supply per pot, the lower dry-matter production of the dry plots caused a higher nitrogen supply per gram of dry matter. This is associated with a higher nitrate content and a lower carbohydrate content. In the low light intensities the nitrogen supply was high both in the dry and in the moist plots In these conditions of similar nitrogen supply the above mentioned increased sugar contents in the dry plots have been found Therefore the higher nitrogen supply, together with the lower growth rate of the dry plots, has effected the interaction of light intensity and water supply on the water-soluble-carbohydrate content mentioned in par 5.1.4. and 5.3.5..

In examining the material of the indoor experiments for the correlation of the crude-protein content with the water-soluble-carbohydrate content, it is found that this correlation is clearly negative, if all treatments are considered together. The regression line corresponds rather well with the one DE ROO et al. (1963) found in 49 different genotypes of tetraploid perennial ryegrass, subjected to the same treatment.

This general picture is, however, composed of different parts. Under influence of the light intensity and the nitrogen supply the correlation of the crude-protein content with the water-soluble-carbohydrate content is highly negative. With a sub-optimal nitrogen supply the correlation is positive under influence of the temperature: because the crude-protein content is low at a high temperature, while the carbohydrate content is low as well. With a liberal nitrogen supply the correlation is somewhat negative (indoor experiment 1963-VI, low light intensity) because in this case no decrease occurs in the crude-protein content, affected by the temperature. Influenced by the water supply, the correlation of the crude-protein content with the carbohydrate content is positive in low light intensity and negative in high light intensity; this is caused by the interaction of light intensity and water supply on the carbohydrate content.

5.4.6. *Crude fibre*

The crude-fibre content is frequently found to be correlated with age and dry-matter yield of the grass (ALBERDA, 1962; VAN BURG, 1962). When the indoor experiments are investigated on this correlation, it is found that there is only a very slight correlation between the crude-fibre content and the dry-matter yield. If the moist and dry treatments are separately considered, no correlation at all is found within each group.

ALBERDA (1963) found that the crude-fibre content in the grass of solitary growing vegetative plants does not rise with the length of the growing period, whereas such an increase does occur in a grass crop. In a recent investigation in the phytotron ALBERDA (1965) once more found that the chemical composition, including the crude-fibre content, calculated on sugar-free dry matter, is not influenced by light intensity and temperature. However, in the experiments described here, the chemical composition of the sugar-free dry matter is influenced by the external conditions.

Therefore the crude-fibre content has to be approximated by another method.

It is found in the first place that the reaction of the crude-fibre content in the fresh matter to the external conditions is frequently different from that of this content in the dry matter.

In the indoor experiments the content in the fresh matter is stimulated by light intensity and water shortage, whereas the content in the dry matter is reduced. Nitrogen fertilization has a reverse effect, but a temperature rise shows a positive influence on the content in the fresh matter as well as on that in the dry matter.

As already mentioned in paragraph 3.2.5., the content of a constituent in the dry matter is equal to its share in the total quantity of all constituents, water excluded. The same holds for the content in the fresh matter, but now water included. However, sometimes the content in the fresh matter is a more suitable quantity in plant physiological explanations than the content in the dry matter. This is shown by the following treatment of the crude-fibre content in the fresh matter.

The crude fibre and rest together comprise all cell-wall constituents (chapter 3), as cellulose, lignin, hemicellulose, pectins, pentosans and also lipids, organic acids and some constituents less important in concentration. The crude fibre mainly consists of the cellulose and a small part of the lignin and hemicellulose; the total remaining non-analysed constituents are considered to make up the rest.

A relatively great part of the cell walls of a grass plant is formed by the vascular bundles, with the important function of transporting water to the leaves. This water is transpired for a great part. BROUWER et al. (1964) have found that transpiration and vascular-bundle area show a close positive correlation. This may also be inferred from SINNOTT's (1960) book: in a high light intensity the vascular-bundle system is more developed than in a low light intensity. A low relative air humidity also stimulates this development. It can also be inferred from the literature that the xermorphic properties in the plant are stimulated by an increasing transpiration. One of these properties is that the cell walls thicken (ALLSOPP, 1965; SINNOTT, 1960).

Very clear correlations are found, if the crude-fibre content in the fresh matter of the moist treatments in the various experiments, as a standard for the vascular-bundle area and the extent of xeromorphy, is plotted against the measured water consumption per gram of fresh matter, as a standard for the transpiration of the leaf (figure 10a).

Each separate part of figure 10a shows that the crude-fibre content in the fresh matter is highly correlated with the water consumption. This water consumption depends on the factors light intensity, temperature and fertilization, but apart from this, these factors do not have a specific effect on the crude-fibre content in the fresh matter.

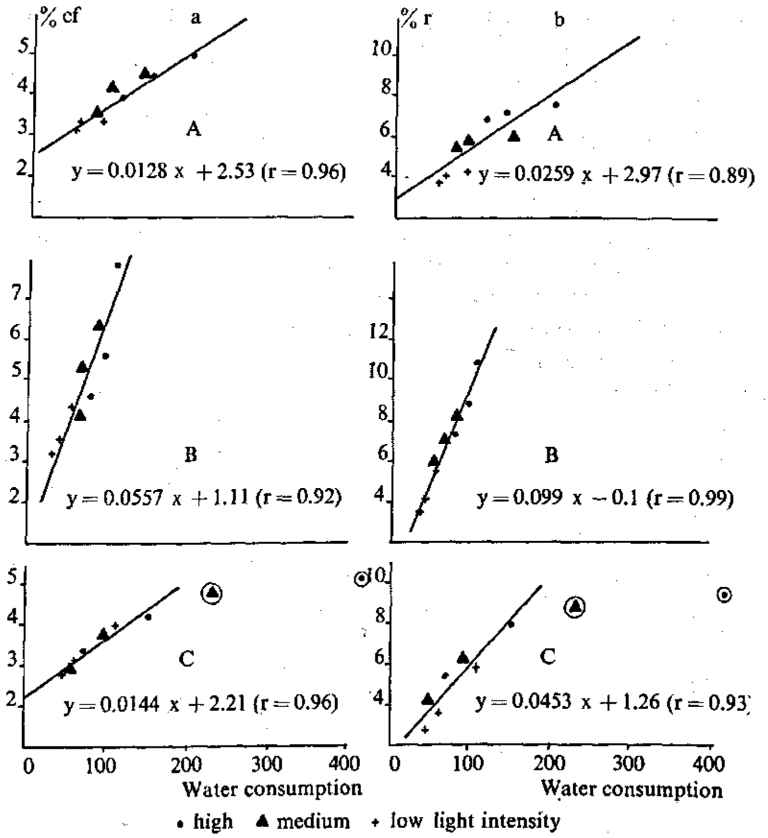


FIG. 10 Relationship between the water consumption per gram of fresh matter and the crude-fibre content (fig. 10a) and the rest content (fig. 10b) of the fresh matter (moist treatments only)
A. Indoor exp. 1963-II
B. Indoor exp. 1963-III
C. Indoor exp. 1964

In the indoor experiment 1964, some points deviated from the linear curve, especially because the calculated water consumption per gram of fresh matter in the normal and high light intensity was high with a low fertilization. The grass of these treatments was very short and the coverage poor, resulting in a great part of the water being evaporated by the soil and not transpired by the grass. These two points were therefore left out of the regression calculation.

The regression lines of the various experiments are not the same. This could neither be expected, because the morphological structure of a shooting crop with many stems is different from that of a non-shooting crop. The fresh weight of the shooting crop is high, but the transpiring area (= leaf area) is relatively small. The stems also contain a relatively high weight of vascular-bundle and supporting tissues. Both factors result in an increased crude-fibre content with the same transpiration compared to vegetative grass.

The number of stems compared to the number of tillers at the beginning of the experiment was determined in the various experiments as a standard for the leaf/stem-ratio. If the regression coefficients of the various experiments are plotted against this percentage of stems, a positive correlation is found (figure 11a). If the leaf/stem-ratio had been determined, probably the correlation of the crude-fibre content in the fresh leaf and the transpiration per gram of fresh leaf would have been the same in all experiments.

Water shortage retards the transpiration per gram of fresh matter, whereas the crude-fibre content in the fresh matter is increased, presumably, by the smaller concentration of water in the grass. The correlation coefficient between the crude-fibre content in the fresh matter and the water consumption per gram of fresh matter in the dry treatments, by the way, is always lower than that in the moist treatments. The reason for this is that it is a practical difficulty to keep the

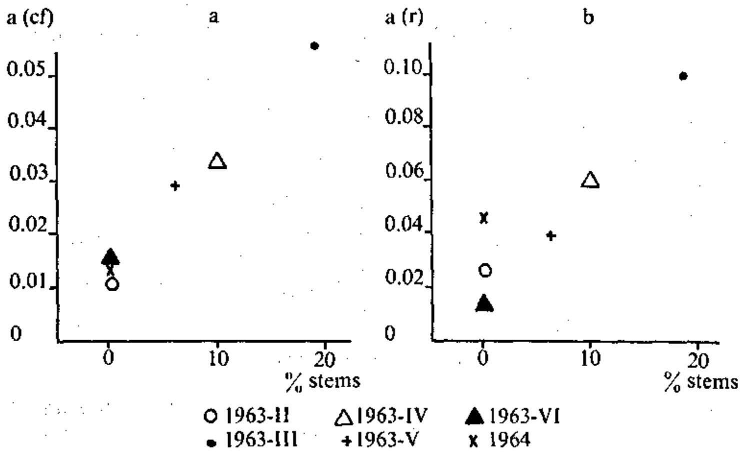


FIG. 11 Relationship between percentage stems and the regression coefficient from fig. 10
a. for the crude-fibre content
b. for the rest content

moisture level of the different treatments at the same level under dry conditions.

Now we return to the observation made at the beginning of this paragraph, that the crude-fibre content in the dry matter reacts differently from the content in the fresh matter. To answer the question how this is possible the fresh matter is closely inspected. It is then found that in the indoor experiments 1963 the crude-protein and ash contents in the fresh matter were hardly influenced by the light intensity and temperature, unlike the crude-fibre and rest contents, which were increased by both. The water-soluble-carbohydrate content rises sharply with an increasing light intensity and decreases with a rising temperature.

The crude-fibre content in the fresh matter is increased with light intensity, but the carbohydrate content is advanced much more, resulting in a sharp increase in the dry-matter content. The share of the crude fibre in the total dry matter is therefore reduced with increasing light intensity and with it the crude-fibre content in the dry matter.

Since the water-soluble-carbohydrate content is reduced by a rising temperature, the dry-matter content hardly increases with the temperature, whereas the crude-fibre content in the fresh matter is increased. The share of the crude fibre in the dry matter increases therefore with temperature, and with it the crude-fibre content.

Nitrogen fertilization often shows the same effect as light shortage. The fertilization reduces the crude-fibre content in the fresh matter. However, the water-soluble-carbohydrate content diminishes to a greater extent and the dry-matter content is also much reduced, resulting in an often increased crude-fibre content in the dry matter.

Water shortage inhibits transpiration and results in a serious xeromorphy, expressed in the thickened cell walls. Because the cells show a greater content of carbohydrates, proteins and similar substances in drought, the dry-matter content is relatively more increased than the crude-fibre content in the fresh matter. The crude-fibre content in the dry matter is therefore reduced.

5.4.7. *Rest*

The rest content in the grass may be approximated in the same way as the crude-fibre content, for the rest consists for a great part of cell-wall constituents, dissolved during the crude-fibre determination, cf paragraph 5.4.6..

Like the crude-fibre content, the rest content is correlated with the transpiration of the grass. This may be seen in figure 10b. Here too, the morphological structure of the plant influences the regression coefficients (figure 11b). The correlation coefficients as a rule are lower than those with the crude fibre. This is not surprising for two reasons:

a. The error made in determining the nitrate, crude-protein, ash, water-soluble-carbohydrate and crude-fibre contents are reflected together in the 'determination error' of the rest content.
b. The rest partly consists of lipids and organic acids which may be influenced by external conditions differently from the cell walls.

The rest content in the dry matter infrequently also shows a correspondence with the water-soluble-carbohydrate content: the rest content is increased with light intensity (all indoor experiments) and decreased by nitrogen application (indoor experiment 1963-VI and indoor experiment 1964). This may be related to the concentration of carbohydrates present under these conditions. According to SINNOTT (1960) a part of the carbohydrates in plants with a high soluble-carbohydrate content is polymerised to hemicellulose, which is deposited on the cell walls.

It is evident from the results of the indoor experiments that the light intensity, temperature, water supply and nitrogen application have a considerable effect on the dry-matter yield and chemical composition of the grass.

In this it was found that the nitrate content is especially determined by the nitrate reduction, which is influenced by the light intensity. The effect of the various factors on the crude-protein and ash contents are closely related to the dry-matter yield and the nitrogen and mineral supply. The water-soluble-carbohydrate content depends on the carbohydrate formation and consumption. The crude-fibre and rest contents are greatly affected by the transpiration of the grass, water-soluble-carbohydrate content and morphological structure of the plant.

6. RESULTS OF THE FIELD EXPERIMENTS

Chapter 5 demonstrated the way in which the factors light intensity, temperature, water supply and fertilization influence the dry-matter production and chemical composition of grass grown in pots. Chapter 6 deals with the way in which these factors affect the grass in experiments with a field crop.

The following experiments are subsequently described:

the field experiments with varied light intensities;

the field experiments of 1962 and 1963 in which the grass was cut during the whole season;

the twenty-five years' experiment in which the same treatment was applied.

After that, all the results will be discussed together in chapter 7.

6.1. Field experiment, spring 1964

This experiment was intended to investigate the influence of the light intensity on the dry-matter production and chemical composition of a closed grass sward. The lay out of this experiment has been described in paragraph 4.3.2..

The results are recorded in table 25; table 26 reflects the effect of 100 cal $cm^{-2}day^{-1}$ of light on the production and chemical composition.

Table 25 shows that the fresh-grass yield is hardly affected by the light intensity. The dry-matter yield of the grass and the stubble are both stimulated by an increased light intensity, that of the stubble to a greater extent than that of the grass. This considerable light effect on the stubble probably is caused by increased tillering in the higher light intensity. Because of the rather high cutting height an important part of these young tillers could not be harvested, and therefore they remained in the stubble.

TABLE 25. Dry-matter production and chemical composition of the grass of the 2 light intensities in the field experiment, spring 1964

	Light intensity	
	88 cal $cm^{-2}day^{-1}$	282 cal $cm^{-2}day^{-1}$
Grass fresh-matter yield (kg a^{-1})	186.4	194.5
Grass dry-matter yield (kg a^{-1})	21.4	26.3
% dm	11.5	13.0
Stubble weight (kg dm a^{-1})	18.4	30.8
% NO_3	0.12	0.04
% cp	19.9	15.6
% ash	13.1	10.8
% wsc	4.1	11.8
% cf	28.5	26.8
% r	34.4	35.0
N-quantity in grass (kg a^{-1})	0.66	0.65
ash-quantity in grass (kg a^{-1})	2.73	2.78

TABLE 26. Effect of 100 cal $cm^{-2}day^{-1}$ on the dry-matter yield and chemical composition of the grass in field experiment, spring 1964

Dry-matter yield (grass) (kg a^{-1})	+2.4
Dry-matter yield (stubble) (kg a^{-1})	+6.2
% NO_3	-0.04
% cp	-2.1
% ash	-1.1
% wsc	+3.9
% cf	-0.9
% r	+0.3

The quantity of nitrogen and ash in the cut grass is hardly affected by the light intensity. Based on the low stubble weight in the low light intensity it is quite possible that the light intensity had a positive influence on the uptake of nitrogen and minerals of the whole plant.

6.2. FIELD EXPERIMENT, AUTUMN 1964

The experiment was carried out in autumn, also to see if there is any correspondence between spring and autumn grass, grown under comparable light intensities.

The results of this experiment mentioned in figure 12 and in appendix 4, on many points correspond well with those of the indoor experiment 1964. Only the dry-matter content deviates. This was because it started to rain during the harvest; treatment L_3N_1 was harvested completely dry, but the other treatments were all to varying extents damp from the rain. Here too, it is striking, that the effect of the light intensity on the chemical composition is much larger in the range from 48 to 240 cal $cm^{-2}day^{-1}$ than in the range from 240 to 547 cal cm^{-2} day^{-1}.

The light effect per 100 cal $cm^{-2}day^{-1}$ is to be found in table 27.

The figures of this experiment again indicate the same direction as the preced-

TABLE 27. Effect of 100 cal $cm^{-2}day^{-1}$ in the range of 100 to 500 cal $cm^{-2}day^{-1}$ on the dry-matter production and chemical composition of the grass in the field experiment, autumn 1964

	25 kg N ha^{-1}	125 kg N ha^{-1}
dm (kg a^{-1})	+1.25	+4.25
% NO_3	-0.15	-0.57
% cp	-2.4	-2.2
% ash	-0.8	-0.8
% wsc	+3.9	+2.6
% cf	-0.7	-0.7
% r	0	+1.0

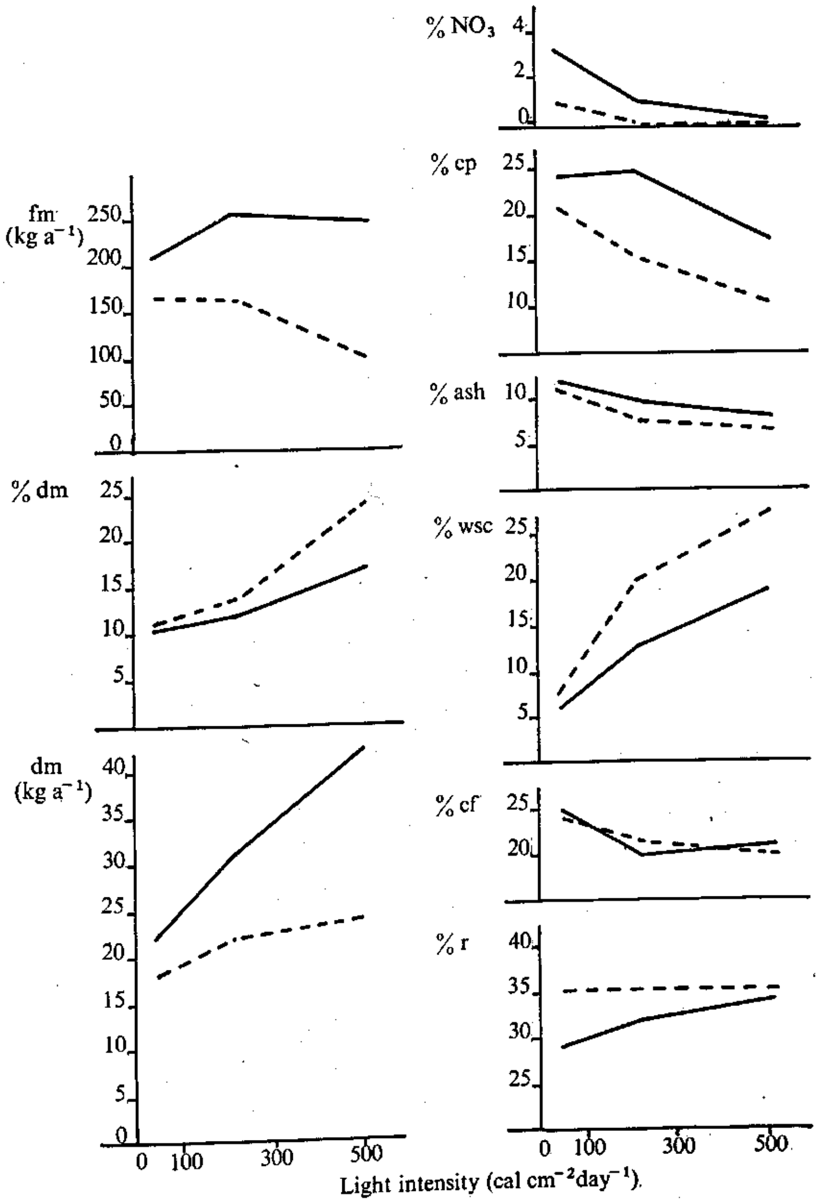


FIG. 12 Production and chemical composition of the grass of the 6 treatments from the field experiment, autumn 1964
(- - - - - 25 kg N/ha, —— 125 kg N/ha)

ing experiment, viz. increased dry-matter yield, and dry-matter content, lower nitrate, crude-protein, ash and crude-fibre contents and higher water-soluble-carbohydrate and rest contents in higher light intensity.

The chemical composition of this non-shooting grass in high light intensity much resembles that of the spring grass (JAGTENBERG, 1961; KLETER, 1961; see paragraph 2.2.2.).

The light effect on the water-soluble-carbohydrate content was higher with 25 kg N/ha than with 125 kg N/ha. With 25 kg N/ha the coverage of the crop was poor and the plants grew more or less separately. With 125 kg N/ha the crop was much denser, especially in the high light intensity. This led to a rather considerable shading effect of the leaves. Only the top leaves receive much light and will form much carbohydrate. In harvesting and sampling all the cut grass is treated and the carbohydrate in the top leaves is distributed over the whole dry-matter production. The slighter light effect at the high nitrogen level may be explained in this way.

The results of this experiment clearly indicate that the low light intensity in autumn is an important reason for the low dry-matter production, fairly high crude-protein and crude-fibre contents and of the rather low nitrogen-free extract content.

6.3. FIELD EXPERIMENTS 1962 AND 1963

The experiments were laid down to collect data on the seasonal trend in the dry-matter production and chemical composition of the grass on farm fields. These experiments also offer the opportunity to investigate to what extent this seasonal trend is dependent on the external conditions. The seasonal trend in this case is defined as the trend, occurring during the growing season in average weather conditions, in the production and chemical composition of the grass with the selected cutting treatment.

Unlike the preceding experiments, in which the factors light intensity and temperature were varied, the grass in these experiments grew under natural conditions of light and temperature. The sole factors varied, were fertilization, sprinkling and cutting treatments.

The influence of the light intensity and temperature in these experiments can only be investigated by comparing different years and subsequent cuts in the growing season.

The following effects are distinguished in the experiments:
the influence of nitrogen fertilization;
the influence of water shortage;
the influence of light intensity and temperature.

6.3.1. *Influence of nitrogen fertilization*

The influence of the nitrogen fertilization, averaged over the sprinkled treatments of the four soil types in 1963, is to be found in table 28.

TABLE 28. Total production and average chemical composition of the grass at the two nitrogen levels of the field experiment 1963

	Nitrogen application(kg $ha^{-1}year^{-1}$)	
	240	560
Fresh-matter yield (kg a^{-1})	674.6	990.2
Dry-matter yield (kg a^{-1})	95.2	122.7
% dm	14.0	12.4
% NO_3	0.07	0.94
% cp	19.0	24.6
% ash	9.8	10.3
% wsc	12.2	8.7
% cf	22.0	22.5
% r	37.0	33.0
N-quantity in grass (kg a^{-1})	2.70	4.76

The effect of the nitrogen fertilization, averaged over the whole year, corresponds well with the expectations: an increased dry-matter production and a lower dry-matter content, higher nitrate, crude-protein and ash contents and lower water-soluble-carbohydrate and rest contents. The crude-fibre content is, however, little influenced. The quantity of nitrogen in the grass is higher by the more liberal nitrogen application. Upwards 60% of the nitrogen, applied in excess on the heavily nitrogen fertilized plots, has been taken up by the grass. This is a percentage also found frequently in nitrogen fertilization experiments.

The seasonal trend is to be found in figure 13. This figure shows, that the nitrogen effect on the dry-matter production varies rather widely. After a harvest with a considerable nitrogen effect, usually one follows with a smaller effect, and the reverse. This is caused by the influence of the nitrogen on regrowth; since the dry-matter production is higher with much nitrogen, the regrowth in certain cases is retarded, which was also found by FRANKENA (1941) and VAN BURG (1960).

The nitrogen effect on the chemical composition is almost the same in the different parts of the growing season, despite the difference in nitrogen application being smaller in autumn than earlier in the season. Only the difference in nitrate content between the nitrogen levels was smaller in the second part of the season than in the first part, but this was to be expected from the difference in nitrogen application.

The four experimental fields showed a few level differences in the dry-matter production and chemical composition of the grass. These differences were found to be caused by differences in the productivity of the swards and in the nitrogen supply of the soil. The seasonal trend in the dry-matter production and chemical composition of the grass did not show any true differences between the experimental fields. The experimental field effect is left therefore out of consideration.

6.3.2. *Influence of water shortage*

In the preceding the results of the sprinkled plots were mentioned. In some

TABLE 29. Dry-matter yield and chemical composition of the grass from the sprinkled and non-sprinkled plots in the highest nitrogen treatment of field experiment 1963 on humus poor sandy soil (harvested on 23 August)

	Sprinkled	Non-sprinkled
Dry-matter yield (kg a^{-1})	12.4	6.4
% dm	11.4	12.0
% NO_3	0.47	0.90
% cp	24.9	28.4
% ash	10.2	12.9
% wsc	5.7	3.6
% cf	23.8	20.4
% r	34.9	33.8

drought periods the effect of water shortage could be observed. An example of this effect is to be found in table 29, which mentions the dry-matter yield and chemical composition of the grass in the highest nitrogen treatment on the light, sandy soil on 23 August 1963.

The drought effect in each case corresponded with that in the indoor experiments 1963 in high light intensity: lower dry-matter yield, higher dry-matter

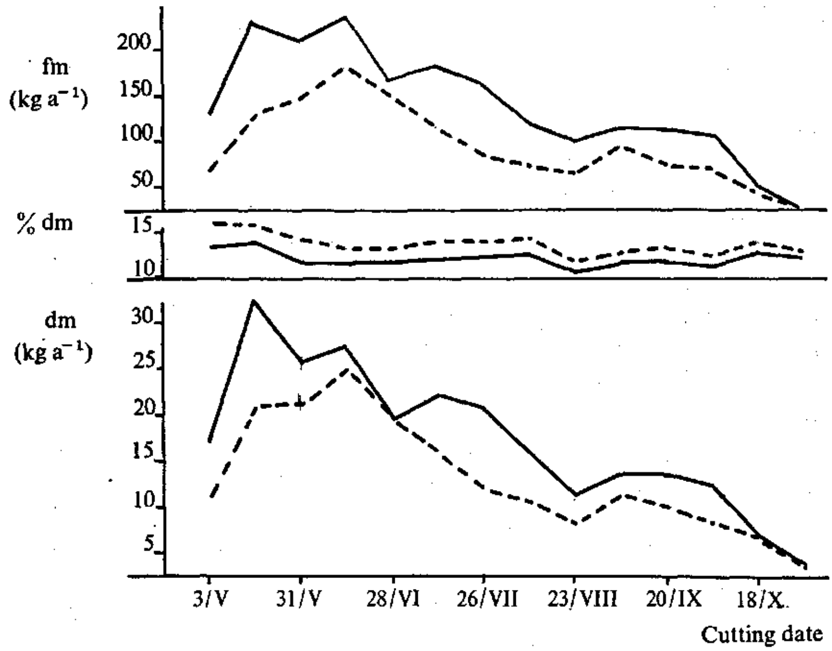
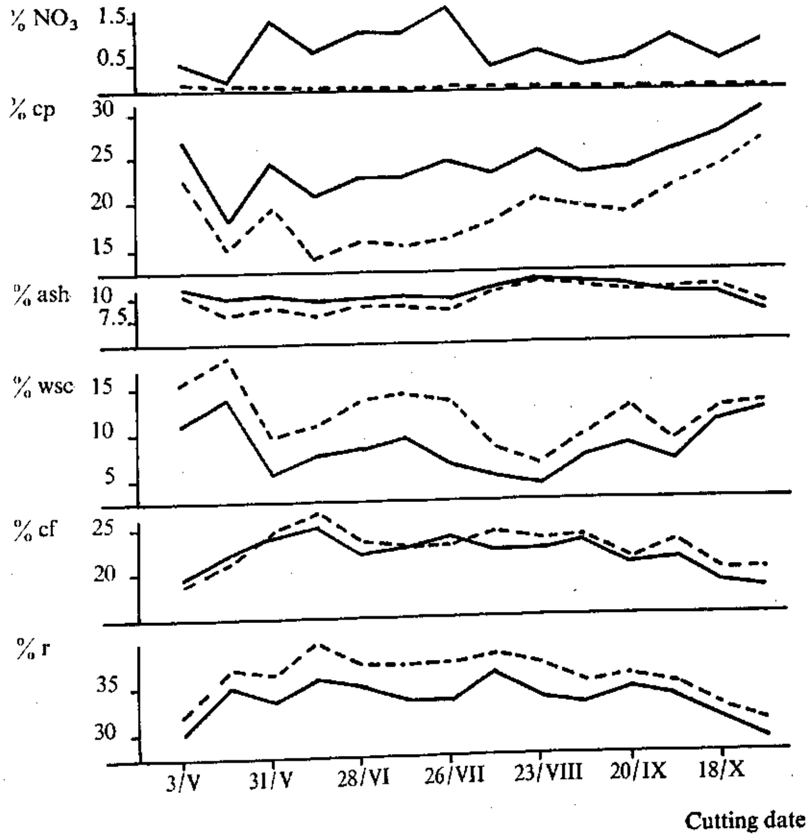


FIG. 13 Seasonal curve of the production and chemical composition of the grass from the field experiment 1963, with high (——) and low (- - - - -) nitrogen fertilization

FIG. 13 continued



content, higher nitrate, crude-protein and ash contents, and lower water-soluble-carbohydrate, crude-fibre and rest contents.

In his sprinkling experiments, VAN BURG (1962) also found a decrease in the dry-matter yields with sometimes higher and sometimes lower nitrate and crude-protein contents and frequently reduced crude-fibre contents. In his sprinkling experiments MAKKINK (1949) found under dry conditions also decreased productions, sometimes higher crude-protein contents and often lower crude-fibre contents.

However, in their ground-water level experiments MAKKINK (1962) and MINDERHOUD (1960) found almost no influence of the ground-water table on the crude-fibre content.

Probably the water supply via the soil, also with the high ground-water tables in the experiments of MAKKINK (1962) and MINDERHOUD (1960), is still insufficient under dry conditions to prevent water shortage, whereas the water supply by sprinkling is completely sufficient in the experiments of MAKKINK

(1949) and VAN BURG (1962). This difference in experimental technique may cause the varying reaction in the crude-fibre content.

6.3.3. *Influence of light intensity and temperature*

In the indoor experiments 1963, the chemical composition of the grass in the different parts of the season reacted similarly to light and temperature. The light effect in the manipulative field experiments almost corresponded with that in the pot experiments. In the experiments dealt with here, the light and temperature effect can only be found by comparing the different cuts, via a regression calculation. The factors light and temperature, moreover, are correlated to some extent. The correlation coefficient of the average light intensity and average temperature in the various four weeks' growing periods in these experiments was + 0.31.

The weather in the growing seasons 1962 and 1963 was almost the same, which was already demonstrated in figure 4. This figure shows that the light intensity was almost the same as the average, but the temperature was below and the precipitation above the average. Periods of drought occurred in June 1962 and in July-August 1963.

The total dry-matter production and the average chemical composition of the grass in both years, like the average weather conditions during the growing season, have been mentioned in table 30.

TABLE 30. Dry-matter yield and average chemical composition of the grass from the field experiments 1962 and 1963 as well as the average weather conditions

	1962	1963
Light intensity (cal $cm^{-2}day^{-1}$)	298	283
24-hours' temperature	12.4	13.1
N-fertilization (kg ha^{-1})	300	400
Dry-matter yield (kg a^{-1})	96.3	108.9
% dm	15.2	13.2
% NO_3	0.06	0.50
% cp	18.0	21.8
% ash	7.6	10.0
% wsc	16.2	10.4
% cf	21.6	22.2
% r	36.5	35.1

This table demonstrates some differences between the years. They are especially caused by the difference in nitrogen fertilization. In 1963 more nitrogen was applied than in 1962 and this led to a higher dry-matter yield, increased nitrate, crude-protein and ash contents, lower water-soluble-carbohydrate and rest contents and somewhat higher crude-fibre content.

The trend in the production and chemical composition of the grass of the sprinkled swards in 1963 is already shown in figure 13. The general trend corresponds rather well with the data from the literature, which have been discussed in paragraph 2.2.2..

To calculate the light and temperature effect on the dry-matter yield and chemical composition, the average yield and chemical composition have been used per harvest date of the sprinkled plots in the years 1962 and 1963. The results of the multiple linear regression calculation are mentioned in table 31.

TABLE 31. Regression equations of the dry-matter yield and chemical composition of the grass with the light intensity in cal cm^{-2} $day^{-1}10^{-2}$ (x_1) and temperature in °C (x_2) of four weeks before the harvest in the field experiments 1962 and 1963

Regression equation	R^2	s
dm (kg a^{-1}) = 5.15 + 7.25 x_1 − 0.90 x_2	0.69	4.0
% cp = 27.9 − 4.20 x_1 + 0.409 x_2	0.63	2.7
% ash = 7.6 − 0.61 x_1 + 0.233 x_2	0.19	1.4
% wsc = 27.6 + 1.51 x_1 − 1.390 x_2	0.48	3.6
% cf = 12.0 + 1.24 x_1 + 0.450 x_2	0.64	1.4
% r = 25.0 + 2.10 x_1 + 0.316 x_2	0.55	2.0

The following may be inferred from this table:

a. The dry-matter production is rather closely correlated to the light intensity and temperature. The temperature effect is, however, negative.

b. The crude-protein and ash contents are also correlated to the light intensity and temperature. The symbols of the regression coefficients are opposite to those which were found for the dry-matter yield. Here again an explanation of the crude-protein and ash contents from the dry-matter yield is obvious.

c. The water-soluble-carbohydrate content is somewhat raised with light intensity and sharply decreased with temperature.

d. The crude-fibre and rest contents are both increased with light intensity and temperature.

In comparing the results of this field experiment (table 31) with those of the indoor experiments (table 24) and the field experiments with light levels (tables 26 and 27), many regression coefficients indicate the same direction, whereas important deviations occur as well. In paragraph 6.4. it will be demonstrated that these deviations are associated with the internal growth rhythm of the grass during the growing season, resulting in cuts with a varying production level and chemical composition. If these cuts are combined, the above-mentioned deviations occur.

6.4. TWENTY-FIVE YEARS' EXPERIMENT 1938–1962

In this experiment different nitrogen fertilization levels and managements have been compared during many years. In such experiments the data may be grouped and studied in various ways.

Apart from the two nitrogen levels five management treatments were applied in this experiment. The management treatments are considered as parallels in this case, because the harvest dates per cut did not vary much. More recent calculations showed, that the nitrogen effect was rather small in these experiments. This is probably associated with the relatively low nitrogen applications. This nitrogen effect is therefore left out of consideration.

The data collected on the dry-matter production and chemical composition with the associated calculated weather conditions are first averaged per cut, per nitrogen level and per year. The results obtained are studied as a whole and arranged per cut, after which the results of both calculations are compared. In the first calculation the seasonal trend in the growth rhythm and its, partly incidental, correlations with light intensity and temperature are important. In treating the same cut in the different years the seasonal trend is more or less eliminated. In this calculation the results may be compared with those of the manipulative experiments.

6.4.1. *Influence of light intensity and temperature on the dry-matter production and chemical composition of grass in all cuts together*

From the entire group of data the correlation of the dry-matter yield and chemical composition with light intensity and temperature was again calculated by a multiple linear regression technique. The results are mentioned in table 32.

TABLE 32. Regression equations of the dry-matter yield and chemical composition of the grass with the light intensity in cal $cm^{-2}day^{-1}10^{-2}$ (x_1) and temperature in °C (x_2) of four weeks before the harvest, of the entire material in the 25-years' field experiment

Regression equation	R^2	s
dm (kg a^{-1}) $= + 13.6 + 5.36\,x_1 - 0.579\,x_2$	0.38	5.20
% cp $= + 24.8 - 2.70\,x_1 + 0.236\,x_2$	0.34	2.80
% ash $= + 8.9 - 0.70\,x_1 + 0.235\,x_2$	0.34	1.06
% cf $= + 17.4 + 1.00\,x_1 + 0.374\,x_2$	0.25	2.13
% nfe $= + 49.0 + 2.30\,x_1 - 0.845\,x_2$	0.41	3.15

The regression equations mentioned in this table correspond well with those in paragraph 6.3.3.. The regression coefficients are almost the same as those in table 31. There only are some level differences. These probably have been caused by the longer growing period of the grass, an average of 41 days compared to 29 days in the field experiment in paragraph 6.3.. The correspondence is all the more remarkable, considering sprinkling did not take place in periods of drought in this twenty-five years' field experiment. The effect of a possible water shortage on the results has apparently been very slight.

There is good correspondence in the response of the dry-matter yield and the chemical composition to light intensity and temperature of both field experiments, in which the various cuts were applied during the growing season. However, in a number of cases they widely deviate from the indoor and field experiments in which the light and temperature effect was investigated per cut.

It has already been mentioned, that this experiment, because of the great number of years, offers the opportunity also to investigate the light and temperature effect per cut. This is discussed in the following paragraphs.

6.4.2. *Influence of light intensity and temperature on the dry-matter production and chemical composition of grass in the separate cuts*

In this investigation the data per cut have been treated in the way described in the preceding paragraph. The regression equations obtained are reflected in figure 14. In this the influence of the light intensity is indicated at a temperature of 15°C, and the influence of the temperature at a light intensity of 300 cal cm^{-2} day^{-1}, resulting in figure 14 being comparable to figure 7. Figure 14 also reflects the regression equations from table 32.

This figure clearly shows, that the variation in the light intensity and temperature per cut has been relatively small, despite the period of upwards 20 years. This small variation probably also led to some of the regression coefficients not being significant.

In the following the regression coefficients of the light intensity and temperature are separately discussed.

6.4.2.1. Influence of light intensity

Figure 14a demonstrates that the regression coefficients of the dry-matter yield with the light intensity in the various cuts correspond well with those in the entire material. They indicate the same trend as in the indoor experiments and in the field experiments with light levels.

What was found for the dry-matter yield also holds for the crude-protein content and to a less extent for the ash content.

The regression coefficient of the crude-fibre content in the first cut corresponds with those from the light-level experiments, those of the other cuts deviate. This deviation was only significant in the second cut, however.

The regression coefficients of the nitrogen-free-extract content correspond in the first and second cut with those from the light-level experiments, the coefficients of the other cuts not being significant.

Summarizing it may be stated that in studying the light effect per cut almost no significant deviations were found compared to the light-level experiments.

6.4.2.2. Influence of temperature

Figure 14b shows that the dry-matter yield in the various cuts, like in the indoor experiments, usually increases with the temperature.

The crude-protein content in the various cuts, like in the indoor experiments, is decreased with the temperature.

In the separate cuts the ash content usually increases somewhat with the temperature, whereas in the indoor experiments negative as well as positive temperature effects were found.

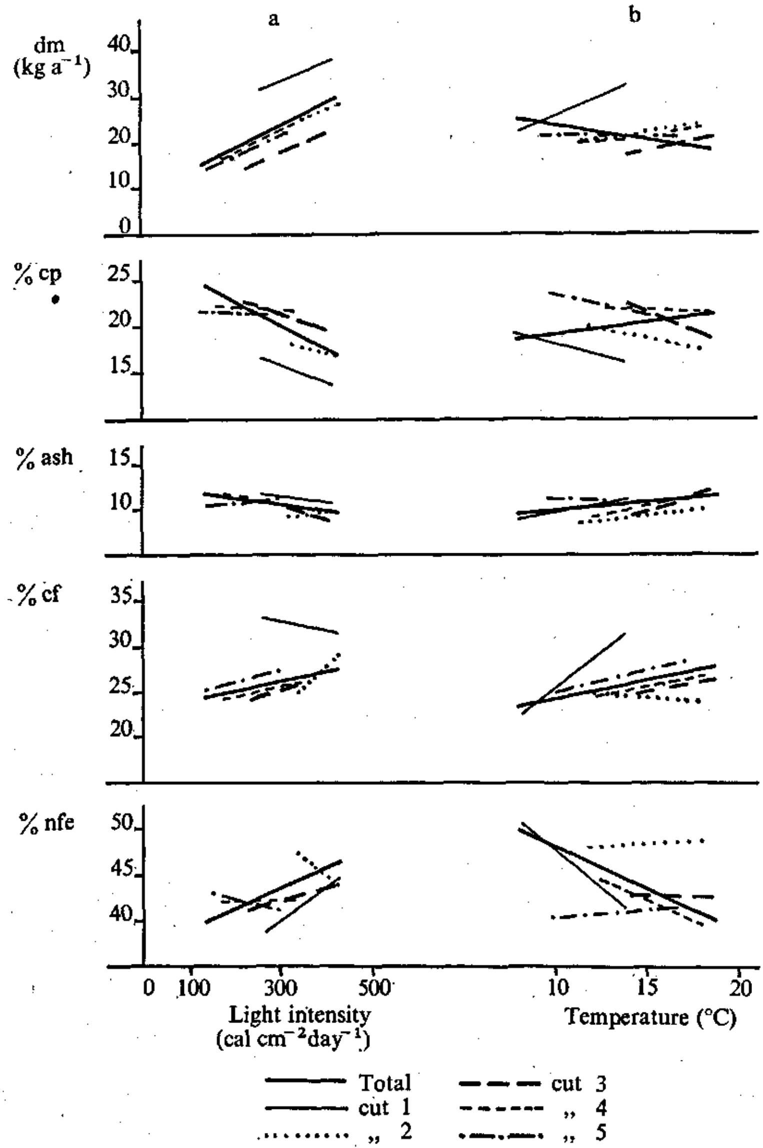


FIG. 14 Effect of light intensity and temperature on the dry-matter production and chemical composition of the grass of the different cuts from the twenty-five years' experiment

The crude-fibre content in most cases increases with temperature, corresponding with the indoor experiments. Only the second cut did not show any effect.

The nitrogen-free-extract content, like in the indoor experiments, was decreased in the first and fourth cuts by the rising temperature, in the other cuts the temperature effect was almost absent.

Summarizing it may be stated that no important differences were established for the temperature effect on the dry-matter production and chemical composition of the grass per cut between the indoor experiments and field experiments.

6.4.2.3. Differences between the cuts

If the results of the five cuts are compared to those of the indoor experiments and the field experiments with light levels, it is striking, that the results of the first cut agree well, but those of the subsequent cuts do not correspond so closely. Yet, based on the indoor and field experiments with light levels, it may be assumed that the light intensity and temperature show the same effect in these following cuts as in the first one. These light and temperature effects are not always found, which, apart from the small variations in light and temperature, may also be the result of differences in the pre-experimental period of the grass. This is only uniform for the first cut; viz. the first cut is always preceded by a period without growth. The second and subsequent cuts are always preceded by a period with growth. This growth differs considerably as a result of light and temperature effects, length of the growing period and management. These factors, via the reserves accumulated in the plant, undoubtedly influence the dry-matter production and chemical composition (DAVIES, 1965).

6.4.3. *Comparison of the light and temperature effect on the grass per cut to that on the entire material*

In the paragraphs 6.4.2.1. and 6.4.2.2. it was shown that the light and temperature effect on the dry-matter production and chemical composition of the grass per cut in this experiment did not essentially differ from that in the experiments with light and temperature levels.

If, however, the light and temperature effect on the grass of the entire material is calculated, clear deviations are found in a number of cases for the light and temperature effect.

These deviations are expressed in figure 14, in which, besides the light and temperature effect per cut, that of the entire material is reflected. Thus the light effect on the crude-fibre content in the grass of all cuts together is positive, but the directly measured light effect is negative. Furthermore, the temperature effect on the dry-matter yield of the entire material is negative, but the effect per cut is positive. The temperature effect on the crude-protein content is just the reverse.

In paragraph 6.4. it was already mentioned that in treating the entire material, next to direct light and temperature effects, the seasonal trend in the growth rhythm, with its incidental correlations with the light intensity and temperature, is important.

Thus in spring the growth rate and the dry-matter production are high and therefore the crude-protein content is low. The temperature is also rather low. The high dry-matter production at the low spring temperatures and the lower production at the higher summer temperatures leads in the entire material to a negative regression coefficient of the temperature with the dry-matter yield. The low crude-protein content at the low spring temperatures and the higher contents at the summer temperatures lead in the entire material to a positive regression coefficient of the temperature with the crude-protein content.

In spring the grass shows a tendency to stem formation. Because of these stems the crude-fibre content is increased, while the light intensity in spring already is rather high. This combination of high light intensity with shooting grass in spring and lower intensity with vegetative grass in late summer and autumn leads in the entire material to a positive regression coefficient of the light intensity with the crude-fibre content.

Thus it is clear that the deviating regression coefficients in the entire material are caused by the specific seasonal trend in the growth rhythm and morphological structure of the grass.

It may be concluded therefore from this experiment that true effects of light intensity and temperature are found, if grass of the same morphological structore is used. However, if data on grass of a different growth rhythm and morphological structure are treated, contradictory results can be expected.

7. GENERAL DISCUSSION AND CONCLUSIONS

In the preceding chapters the different experiments have been separately discussed and the results incidentally compared. In this chapter all the experimental results are compared. Afterwards it will be investigated, with the regression equations obtained, which chemical composition may be expected in the grass in some climatic types and to what extent the chemical compositions calculated agree with those found.

7.1. Comparison of all the experimental results

In the following it will be investigated if the results of all the experiments may be summarized in one general picture. In this the dry-matter production and separate constituents in the dry matter are subsequently treated.

It is stressed again that the growing period in the present experiments always was four weeks.

7.1.1. *Dry-matter production*

In general it holds for all experiments, that the dry-matter yield is higher with an increasing light intensity, within the entire range from 25 to 550 cal $cm^{-2}day^{-1}$. However, the indoor experiments do show that the light effect is smaller above 300 cal $cm^{-2}day^{-1}$ than below this limit. This agrees with the results of the photosynthesis measurements (GAASTRA, 1962), because light saturation for photosynthesis is achieved at about 300 cal $cm^{-2}day^{-1}$.

Nitrogen application also has a positive effect on the dry-matter production. Apart from this there is a positive interaction between the light intensity and the nitrogen application. In the indoor experiments as well as in the field experiments the nitrogen effect was greater in a high light intensity than in a low one. In a very low light intensity incidentally the lowest nitrogen level was already sufficient to procure a maximal production.

In all experiments it was found that the dry-matter yield increased with the temperature. In the present experiments, the temperature range varied between about 10° and 23 °C, and the grass species *Lolium perenne* L. was generally used. Of course in other temperature ranges and with other grass species different temperature effects may be found.

In the indoor as well as in the field experiments it was found that the dry-matter production of shooting grass was higher than that of non-shooting grass.

7.1.2. *Nitrate*

In the present experiments, like in many others mentioned in the literature, it was found that the nitrate content rises with increasing nitrogen applications. It was also established that the nitrate content decreased by dilution with a higher dry-matter production.

In higher light intensity the dry-matter production is increased and the nitrate content is decreased. In addition the specific influence of the light intensity on the nitrate reduction is mentioned here. This always caused very low nitrate contents in a high light intensity, even with a very high nitrogen application. Table 33 demonstrates this once more, mentioning data on the treatments with high nitrogen fertilization of the field experiment in the autumn of 1964.

TABLE 33. Effect of the light intensity (cal $cm^{-2}day^{-1}$) on the nitrate content in the grass of the field experiment, autumn 1964 (% of dry matter)

Light intensity	NO_3
48	3.37
240	1.06
547	0.22

7.1.3. *Crude protein*

In all experiments the crude-protein content was closely related to the dry-matter production. If this dry-matter production is increased with the light intensity, temperature or water supply, a proportional increase in the nitrogen uptake does not occur and the crude-protein content will be reduced. This decrease, influenced by the light intensity, also occurs with very high nitrogen applications.

The nitrogen uptake with high nitrogen application increases more than the dry-matter production and therefore the crude-protein content rises.

In a higher light intensity the dry-matter yield of root and stubble is increased. Nitrogen is also stored in these parts, resulting in less nitrogen being available to the grass. This led to a crude-protein content, which was lower than might be expected from the dry-matter production.

7.1.4. *Ash*

Like in the literature, it was always found that, influenced by the light intensity, water supply and nitrogen fertilization, the ash content runs parallel to the crude-protein content. Unlike the temperature effect on the ash content, which was positive in most cases, whereas this effect on the crude-protein content was usually negative.

7.1.5. *Water-soluble carbohydrate*

The water-soluble-carbohydrate content rises with an increasing light intensity. This increase has been found in all experiments described here. In the indoor experiments the light effect was, however, larger than in the field experiments. This undoubtedly is caused by the light in the indoor experiments falling all around the plants, so that all leaves received the light, whereas in the field experiments the crop grew very dense and only a part of the leaves received full light.

In the present experiments, like in others (cf. ALBERDA, 1965) the nitrogen application always had a reducing effect on the carbohydrate content.

The temperature has a negative effect on the carbohydrate content. This was also found in these experiments.

In paragraph 5.4.5. the correlation of the nitrate content with the water-soluble-carbohydrate content was discussed. This correlation is found in the material of the indoor experiments as well as in that of the high nitrogen levels in the field experiments 1963 and the field experiment, autumn 1964. The curves reflecting the correlation of these contents about coincide in both experiments. The curves are, however, not the same as those found in ALBERDA's (1965) experiments with a liberal nitrogen supply. In the same paragraph it has already been mentioned, that this difference is probably caused by the lower light intensity and the shorter growing period in ALBERDA's experiments.

In the indoor experiments there is almost no influence of the temperature on the curve. In the field experiments 1963 a temperature effect is found with the high nitrogen level: at a higher temperature the curve moves to a lower carbohydrate content. This temperature effect does correspond with ALBERDA's results (1965).

7.1.6. *Crude fibre*

In the literature it can be found that the crude-fibre content decreases with increasing light intensity. This is also found in the experiments discussed here, in the indoor experiments as well as in the field.

In general the crude-fibre content increases with the temperature. This was also found in these experiments. Nitrogen fertilization reduces the crude-fibre content in young grass; in old grass this content is increased. In the experiments described here, these negative or positive effects of nitrogen fertilization were found infrequently. This is probably caused by the growing period of four weeks, resulting in the grass being neither young nor old.

In paragraph 5.4.6. it was proved that the crude-fibre content in the fresh matter is closely related to the water consumption per gram of fresh matter. This relationship could not be studied in the field experiments for two reasons:

a. though the grass was harvested early in the morning, the adhering water varied from cutting date to cutting date. The content of plant physiologically active water and the fresh-matter yield based on this are therefore difficult to determine;

b. the water consumption of the grass was not measured.

7.1.7. *Rest*

The rest comprises the total of the not directly analysed substances in the grass. Little is known until now about the content of this group of substances in grass. Only ALBERDA (1959) mentions some data.

In the various experiments mentioned here, the rest content in the dry matter is in most cases increased with light intensity or temperature. The content is decreased with nitrogen fertilization and water shortage.

The rest consists for a great part of cell-wall substances dissolved during the crude-fibre determinations. Similar as with the crude-fibre content there is a clear positive correlation between the rest content in the fresh matter and the water consumption per gram of fresh matter. There is therefore also a positive correlation between the crude-fibre + rest content in the fresh matter and the water consumption per gram of fresh weight. Since both constituents are correlated with this water consumption, their contents in the fresh matter are interrelated as well.

In the dry matter too, the crude-fibre and rest contents are often positively correlated, being e.g. influenced by the temperature (figures 5 and 7), water supply (figures 5 and 7) and the length of the growing period (table 2).

The rest partly consists of hemicellulose, a polymer of glucose. SINNOTT (1960) states that much hemicellulose is formed, especially with a high sugar content. It is therefore not surprising that in some cases positive correlations are found between the water-soluble-carbohydrate content and the rest content; these correlations occur, influenced by light intensity (figures 5 and 7) and nitrogen fertilization (table 28).

7.1.8. *Nitrogen-free extract*

The nitrogen-free-extract content is the sum of the water-soluble-carbohydrate, the nitrate and the rest contents. Based on the data on both constituents separately, it is clear that the nitrogen-free-extract content is raised by increasing light intensity (table 32) and reduced by a rising temperature (table 32), nitrogen fertilization (table 28) and water shortage (figure 7).

The results of all experiments show that the direct influence of light intensity is almost always of the same size; the same holds for temperature, water supply and nitrogen fertilization.

It is therefore possible to express this final result for the light intensity and temperature quantitatively in a table, reflecting the effect of 100 cal $cm^{-2}day^{-1}$ and of 1 °C on the dry-matter production and the chemical composition of the grass per cut.

Proceeding from figure 14b and table 24 this can be done for the temperature effect; for the light intensity table 24 is less suitable, because the light effect was measured by more or less solitary growing plants (see paragraph 7.1.5.). The results of table 26 and 27 and figure 14a are therefore preferred. The obtained light and temperature effects are mentioned in table 34.

7.2. Influence of the growth rhythm of the grass on the seasonal trend in the dry-matter production and the chemical composition

The regression coefficients from table 31 and table 34 enable now the seasonal

TABLE 34. General effect of 100 cal $cm^{-2}day^{-1}$, and 1 °C on the chemical composition of a grass crop, with a growing period of four weeks

	Light effect	Temperature effect
dm ($kg\ a^{-1}$)	+5.0	+0.70
% cp	- 2.0	- 0.30
% ash	- 1.0	+0.05
% wsc	+3.0	- 0.50
% cf	- 1.0	+0.45
% r	+1.0	+0.30

trend in the dry-matter production and the chemical composition under the average climatic conditions in the Netherlands to be calculated in two ways. The first method is to apply the regression coefficients in table 34, assuming that the light intensity and temperature are the sole factors determining this trend; the second method is to proceed from the seasonal trend found in the field experiments 1962 and 1963, calculating this trend with the regression coefficients in table 31. In the second method the specific seasonal trend in the growth rhythm, not determined by the light intensity, temperature or water supply, is an important factor.

The difference in these two groups of curves may indicate this specific seasonal trend. Figure 15 reflects these curves.

To make both groups more comparable, the constants in the regression equations were so selected, that the chemical composition of the grass was the same at the average light intensity and temperature during the growing season (330 cal $cm^{-2}day^{-1}$ and 14 °C between 15 April and 15 October). To obtain a reliable level for the curves this chemical composition has been chosen equal to the estimated average composition of the pasture grass in the Netherlands (table 35, computed from KLETER, 1961). For the dry-matter production, the constant

TABLE 35. Estimated average composition of the pasture grass in The Netherlands

% cp	18
% ash	12
% wsc	11
% cf	24
% r	35
% nfe	46

in the regression equation from table 34 was so selected, that the calculated average production was equal to that from table 31 at the average light intensity and temperature.

The seasonal trend in figure 15 has been obtained by applying the many-years' average curves from figure 4 in the regression equations.

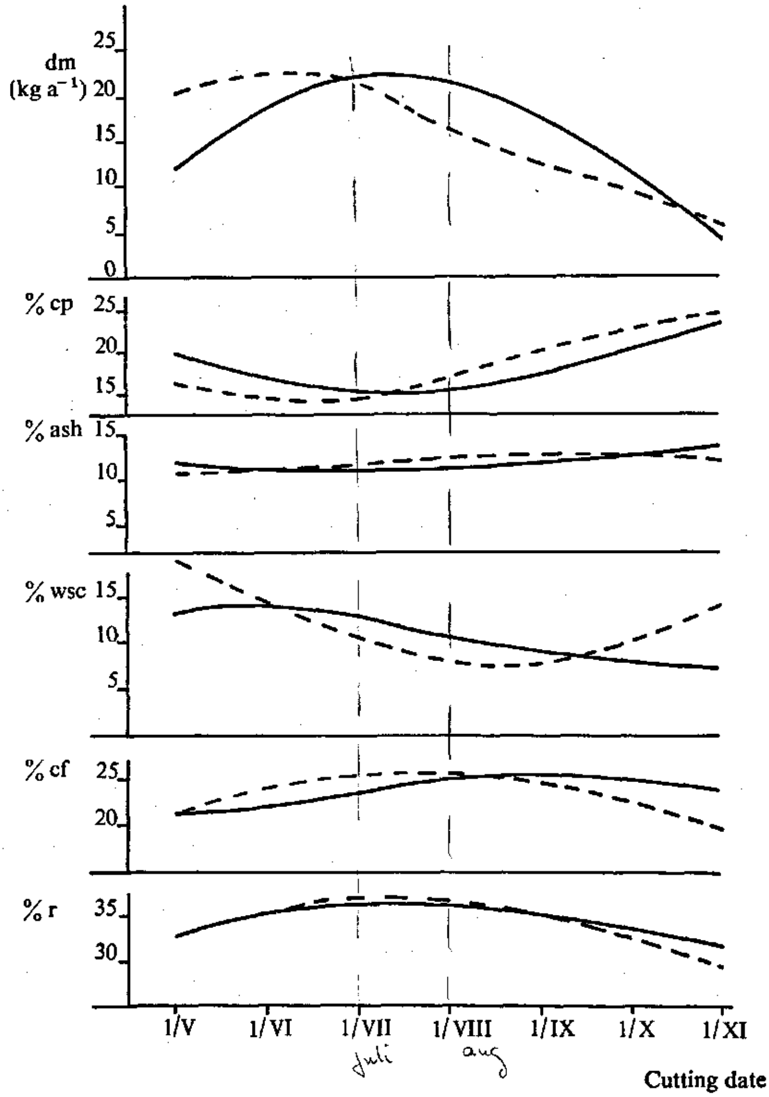


FIG. 15 Seasonal curves of the dry-matter production and chemical composition of grass
a. solely based on light intensity and temperature effects (——)
b. computed from the field experiments 1962 and 1963 (- - - - -)

Figure 15 first shows that the grass should have had its greatest productivity in the month of July. Actually this maximum moved to May, influenced by the shooting and the following mid-summer depression; the productivity remains retarded until autumn.

In accordance with the findings in the dry-matter production, the crude-protein content is lower in spring and higher in summer and autumn than the content calculated from the light intensity and temperature. The ash content is only slightly influenced by the climate and growth rhythm.

According to the calculated curve the water-soluble-carbohydrate content should undergo a slight increase until June, gradually decreasing afterwards from 14 to 7%. Actually this content showed a sharp decrease from 18% at the beginning of May to 7% in August, with afterwards an increase to 14% in autumn. This deviating response is caused by the considerable temperature effect on the carbohydrate content in the field experiments 1962 and 1963. This great effect is presumably the result of the positive correlation of the temperature with the crude-protein content in the field experiment, whereas the correlation was negative in the indoor experiments. Usually the crude-protein content is negatively correlated to the carbohydrate content. If the crude-protein content is included as an independent variable in the regression calculation, the temperature effect on the carbohydrate content may be calculated at a constant crude-protein content. In doing this a regression coefficient of about –0.7 for the relationship between the temperature and the carbohydrate content was found in the indoor experiments as well as in the field experiments 1962 and 1963.

The specific growth rhythm of the grass led to a positive regression of the temperature with the crude-protein content and this positive regression is the reason for the highly negative temperature effect on the carbohydrate content. The seasonal curve of the carbohydrate content found mainly originates therefore from the specific seasonal trend in the dry-matter production.

Low values were calculated for the crude-fibre content in early spring; during the summer it should have increased slowly up to a maximum in September with a slight decrease afterwards. Actually the crude-fibre content is low in spring, but stem formation leads to a rapid increase up to a maximum in July. Thereafter it is rapidly reduced to low values in autumn, probably because of the absence of stems.

No influence of the growth rhythm was found on the rest content. Both curves show that the content is low in spring and autumn and high in summer.

7.3. Comparison of the chemical composition of Dutch grass and tropical grass

Proceeding from the average chemical composition of the grass in the Netherlands, it is possible to calculate, with the figures in table 34, the chemical composition of the grass in various regions of the earth and to compare them with the chemical composition found.

Surinam in the humid tropics has been selected for an example. The results of these calculations are mentioned in table 36, together with the chemical composition found by DIRVEN (1963).

This table shows that in the higher light intensity and temperature of Surinam the calculated average chemical composition of the grass widely deviates from that found in The Netherlands, but corresponds rather well with the chemical composition found there. These results may indicate that the low crude-

TABLE 36. Chemical composition of the grass in The Netherlands and Surinam

	Netherlands	Surinam	
Light intensity (cal cm^{-2}day^{-1})	330	450	
Temperature (°C)	14.3	27.5	
	found	calculated	found (DIRVEN)
% cp	18	11.6	9.8
% ash	12	11.5	10.6
% wsc	11	8.0	
% cf	24	28.8	32.1
% r	35	40.1	
% nfe	46	48.1	47.5

protein content and high crude-fibre content in the grass of Surinam mainly originate from the light intensity and temperature prevailing in this region. The crude-protein and ash contents found are lower and the crude-fibre content higer than the calculated contents in the grass. This may be caused by the generally lower leaf percentage in the tropical grasses (DIRVEN, 1966).

The close correspondence between the calculated and found chemical composition in tropical grass has led to an informative investigation into the influence of light intensity and temperature on the chemical composition of the tropical grass species *Brachiaria ruziziensis* GERMAIN et EVERARD. The preliminary results (DEINUM et al., 1967; DEINUM, 1966) indicate that the effect of light intensity and temperature on the chemical composition of *Brachiaria ruziziensis* and *Lolium perenne* is almost the same. It has also been found that at the same light intensity and temperature the chemical composition in the grass of both species is almost the same.

The nutritive value of tropical grass is in general low. DONALD (1964) suggests this is caused by the rapid growth rate of the tropical grasses at the high light intensities and temperatures. The light intensity in the humid tropics generally is only little higher than in the temperate regions during summer. The temperature is, however, much higher in the tropics.

This high temperature probably is the cause of the high crude-fibre content and, together with the low nitrogen supply, also of the low crude-protein content and with it of the low nutritive value of grass in the tropics.

SUMMARY

The influence of the light intensity, temperature, water supply and nitrogen fertilization on the production and chemical composition of grass has been investigated in a number of indoor and field experiments. These investigations have mainly been carried out with *Lolium perenne* L., in a growing period of four weeks. The following has been determined: fresh-matter yield, dry-matter yield, dry-matter content, and in the dry matter the contents of nitrate, crude protein, ash, water-soluble carbohydrate and crude fibre. The 'rest' content is calculated from $\% r = 100 - \% NO_3 - \% cp - \% ash - \% wsc - \% cf$.

The following was found both in the indoor and in the field experiments:

In the range of 25 – 550 cal $cm^{-2}day^{-1}$ the light intensity shows a positive effect on the dry-matter yield, the dry-matter content and the contents of water-soluble carbohydrate and rest, while a negative influence is found on the contents of nitrate, crude protein, ash and crude fibre (paragraphs 5.1.1., 6.1. and 6.2.).

In the range of 10–23 °C the dry-matter yield increases somewhat with a rising temperature; the dry-matter content hardly changes; the crude-protein and water-soluble-carbohydrate contents diminish and so does the ash content sometimes, but the crude-fibre and rest contents are raised (paragraph 5.1.2.).

Since in all experiments the light intensity and temperature showed almost the same effect on the chemical composition of the grass, their influence could be clearly reflected quantitatively (table 34).

Water shortage decreases the dry-matter yield and advances the dry-matter content, like the nitrate, crude-protein and ash contents. The water-soluble-carbohydrate, crude-fibre and rest contents are usually reduced. However, in low light intensity the carbohydrate content is increased (paragraphs 5.1.3., 5.1.4., and 6.3.2.).

Nitrogen fertilization stimulates the dry-matter yield and reduces the dry-matter content; the nitrate, crude-protein and ash contents are always advanced, just as sometimes the crude-fibre content, whereas the water-soluble-carbohydrate and rest contents are decreased. Light and nitrogen both increase the dry-matter yield, but counteract each other in the chemical composition (paragraphs 5.2., 6.2. and 6.3.1.).

In these experiments the dry-matter production and crude-fibre content of the shooting grass was higher than of the non-shooting grass, but the nitrate, crude-protein and ash contents were lower; the water-soluble-carbohydrate content was hardly influenced (paragraphs 5.3.1. and 6.4.3.).

In the discussion the following could be established:

The nitrate reduction is considerable in high light intensity. In these experiments with often very high light intensities the trend of the curve, indicating the relation of the nitrate content with the water-soluble-carbohydrate content, is

therefore completely different from that in the experiments with fairly low light intensities (paragraphs 5.4.2. and 5.4.4.).

The crude-protein and ash contents may be inferred from the quotients of the nitrogen and mineral uptake of the plant and the dry-matter yield. In this the effect of the light intensity, temperature and water supply on these contents hardly depends on the application rate of nitrogen and minerals (paragraphs 3.3.2., 5.4.3. and 5.4.4.).

The crude fibre and rest consist of cell-wall substances. Considerable amounts of cell wall occur in the vascular bundles; these function in the water transport. With high transpiration the plant shows xeromorphic properties, in which the cell walls are probably thickened. This results in positive correlations of the crude-fibre and rest contents in the fresh matter with the water consumption per gram of fresh matter (fig. 10). External factors affect this water consumption. Since these factors also influence the dry-matter content, viz. by affecting the carbohydrate content in the fresh matter, the crude-fibre and rest contents in the dry matter often react completely different on the external conditions from their contents in the fresh matter (paragraphs 5.4.5. and 5.4.6.).

In the field experiments the light and temperature effects already mentioned were found, if these factors were varied and if their influence was studied per separate cut during a long series of years. However, if their influence was studied in all cuts together, sometimes sharply deviating effects were found. These deviations were caused by the specific, not light intensity and temperature dependent, seasonal trend in the growth rhythm of the grass. Thus a negative correlation was found of the temperature with the dry-matter yield; this was caused by the high dry-matter production at the relatively low temperature in spring. This high production led to a low crude-protein content, resulting in a positive correlation of the temperature and the crude-protein content. In addition the correlation of the light intensity with the crude-fibre content was positive, resulting from the considerable amount of stems in the grass in the relatively high light intensity in spring (paragraphs 6.3.3. and 6.4.).

Finally, informative experiments with *Brachiaria ruziziensis* GERMAIN et EVERARD showed that in this tropical grass effects of light intensity and temperature occur similar to those in *Lolium perenne* L.. It is suggested therefore, that the high temperature, together with the low nitrogen supply, may be the chief explanation for the low nutritive value of grass in the tropics (paragraph 7.3.).

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SAMENVATTING

In een aantal kas- en veldproeven werd de invloed van de lichtsterkte, de temperatuur, de watervoorziening en de stikstofbemesting op de produktie en de chemische samenstelling onderzocht. Deze onderzoekingen zijn voornamelijk uitgevoerd met *Lolium perenne* L., bij een groeiperiode van 4 weken. Hierbij is bepaald: de verse-stofopbrengst, de droge-stofopbrengst, het droge-stofgehalte, en in de droge stof de gehalten aan nitraat, ruw eiwit, as, wateroplosbaar koolhydraat en ruwe celstof, terwijl het zogenaamde rest-gehalte is berekend als $\%\ r = 100 - \%\ NO_3 - \%\ re - \%\ as - \%\ wok - \%\ rc$.

Zowel in de kasproeven als in de veldproeven is het volgende gevonden:

In het trajekt van 25–550 cal $cm^{-2}dag^{-1}$ heeft de lichtsterkte een positieve invloed op de droge-stofopbrengst, het droge-stofgehalte en de wateroplosbaar-koolhydraat- en restgehalten, terwijl een negatieve invloed is gevonden op de gehalten aan nitraat, ruw eiwit, as en ruwe celstof (par. 5.1.1., 6.1. en 6.2.).

In het trajekt van 10–23 °C neemt bij stijging van de temperatuur de droge-stofopbrengst iets toe, terwijl het droge-stofgehalte nauwelijks wordt beïnvloed. De gehalten aan ruw eiwit en wateroplosbaar koolhydraat worden onder deze omstandigheden verlaagd, evenals soms het asgehalte, terwijl het ruwe-celstofgehalte en het restgehalte worden verhoogd (par. 5.1.2.).

Aangezien in alle proeven de lichtsterkte en de temperatuur steeds vrijwel dezelfde invloed hadden op de chemische samenstelling van het gras, kon hun effekt duidelijk kwantitatief worden weergegeven (table 34).

Door watergebrek wordt de droge-stofopbrengst verlaagd, en het droge-stofgehalte verhoogd, evenals het nitraat-, ruw-eiwit- en asgehalte. Het wateroplosbaar-koolhydraat-, ruwe-celstof- en restgehalte worden daarentegen meestal verlaagd. Bij lage lichtsterkte wordt het koolhydraatgehalte echter verhoogd (par. 5.1.3., 5.1.4. en 6.3.2.).

Stikstofbemesting verhoogt de droge-stofopbrengst en verlaagt het droge-stofgehalte; het nitraat-, ruw-eiwit- en asgehalte worden steeds verhoogd, evenals soms het ruwe-celstofgehalte, terwijl het wateroplosbaar-koolhydraat- en restgehalte worden verlaagd. Licht en stikstof verhogen beide de droge-stofopbrengst, maar werken steeds tegengesteld op de chemische samenstelling (par. 5.2., 6.2. en 6.3.1.).

In deze proeven was de droge-stofproduktie en het ruwe-celstofgehalte van schietend gras steeds hoger dan dat van niet schietend gras, terwijl de nitraat-, ruw-eiwit- en asgehalten lager waren; het wateroplosbaar-koolhydraatgehalte werd niet beïnvloed (par. 5.3.1. en 6.4.3.).

In de discussie kon het volgende worden aangetoond:

Bij hoge lichtsterkte is de nitraatreduktie groot. Daardoor is in deze proeven

met dikwijls hoge lichtsterkten het verloop van de curve, die het verband weergeeft tussen het nitraatgehalte en het wateroplosbaar-koolhydraatgehalte, geheel anders dan in proeven met geringe lichtsterkten (par. 5.4.2. en 5.4.4.).

Het ruw-eiwitgehalte en het asgehalte zijn te beschouwen als de quotiënten van de stikstof- en mineralenopname van de plant en de droge-stofopbrengst. Daarbij blijkt de invloed van de lichtsterkte, de temperatuur en de watervoorziening op deze gehalten weinig afhankelijk te zijn van de mate van voorziening met stikstof en mineralen (par. 3.3.2., 5.4.3. en 5.4.4.).

De ruwe celstof en de rest omvatten de celwandbestanddelen. Grote hoeveelheden celwand komen voor in de vaatbundels; deze dienen onder andere voor watertransport. Bij hoge transpiratie vertoont de plant xeromorfe eigenschappen, waarbij de celwanden waarschijnlijk zijn verdikt. Daarom is het verband van het ruwe-celstof- en restgehalte in de verse stof met het waterverbruik per gram verse stof positief (fig. 10). Hierbij hebben de uitwendige faktoren invloed op dit waterverbruik. Omdat deze faktoren tevens invloed hebben op het droge-stofgehalte, onder andere door beïnvloeding van het koolhydraatgehalte in de verse stof, reageren het ruwe-celstofgehalte en het restgehalte in de droge stof vaak geheel anders op de uitwendige omstandigheden dan hun gehalten in de verse stof (par. 5.4.5. en 5.4.6.).

In de veldproeven werden de reeds genoemde invloeden van de lichtsterkte en de temperatuur gevonden, als deze factoren werden gevarieerd en als hun invloed werd onderzocht per afzonderlijke snede gedurende een lange reeks van jaren. Werd hun invloed bestudeerd in alle sneden te zamen, dan werden soms sterk afwijkende effecten gevonden. Deze afwijkingen werden veroorzaakt door het specifieke, niet door de lichtsterkte en de temperatuur bepaalde seizoensverloop in het groeiritme van het gras. Zo werd een negatief verband gevonden tussen de temperatuur en de droge-stofopbrengst; dit werd veroorzaakt door de hoge droge-stofopbrengst bij de vrij lage temperaturen van het voorjaar. Deze hoge produktie leidde tot een laag ruw-eiwitgehalte; daardoor was de korrelatie tussen de temperatuur en het ruw-eiwitgehalte positief. Evenzo was de korrelatie tussen de lichtsterkte en het ruwe-celstofgehalte positief, als gevolg van het hoge stengelaandeel van het gras bij de betrekkelijk hoge lichtsterkte van het voorjaar (par. 6.3.3. en 6.4.).

Ten slotte hebben oriënterende proeven met *Brachiaria ruziziensis* GERMAIN et EVERARD aangetoond, dat bij deze tropische grassoort soortgelijke invloeden van de lichtsterkte en de temperatuur voorkomen als bij *Lolium perenne* L.. Hieruit is te konkluderen, dat de hoge temperatuur en de geringe stikstofvoorziening voor een belangrijk deel een verklaring kunnen geven voor de geringe voederwaarde van gras in de tropen (par 7.3.).

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APPENDIX 1.

LIST OF EXPERIMENTS WITH THEIR TREATMENTS

Experiment	Treatments	Number of replicates
Indoor experiments 1963	6 sub experiments 3 light intensities 3 temperatures 2 moisture levels	5
Indoor experiment 1964	3 light intensities 3 fertilization levels	5
Field experiment 1962	2 soils 3 cutting treatments 6 cutting dates	3
Field experiment 1963	4 soils 2 nitrogen levels 2 moisture levels 2 cutting treatments 7 cutting dates	2
Field experiment, spring 1964	2 light intensities	8
Field experiment, autumn 1964	3 light intensities 2 nitrogen levels	6
25-years' experiment 1938–1962	2 nitrogen levels 6 management treatments 4–6 cutting dates	4

APPENDIX 2.

INDOOR EXPERIMENT 1963-II

	g dm pot^{-1}	% dm	% NO_3	% cp	% ash	% wsc	% cf	% r
moist treatments								
L_3t_3	12.4	22.1	0.05	14.6	6.9	21.8	22.5	34.1
L_2t_3	11.4	18.0	0.08	18.2	8.3	14.4	24.7	34.3
L_1t_3	10.3	13.6	1.51	22.2	11.6	7.3	25.2	32.2
L_3t_2	12.1	21.3	0.06	14.0	5.2	25.8	20.7	34.2
L_2t_2	11.5	17.8	0.10	17.1	7.7	18.6	23.4	33.1
L_1t_2	9.9	13.2	1.49	22.5	11.0	8.5	25.3	31.2
L_3t_1	9.8	21.1	0.06	16.3	5.8	26.4	18.8	32.6
L_2t_1	9.0	17.2	0.10	20.2	7.9	19.2	20.5	32.1
L_1t_1	7.3	13.8	2.32	26.4	10.8	9.8	22.7	28.0
dry treatments								
L_3t_3	6.6	25.0	0.65	22.2	8.8	17.6	19.6	31.1
L_2t_3	7.1	23.5	1.00	23.4	8.7	16.9	20.8	29.2
L_1t_3	5.3	19.1	4.61	26.3	10.8	10.5	22.5	25.3
L_3t_2	7.2	27.5	0.29	21.0	7.5	19.8	18.5	32.9
L_2t_2	7.1	25.4	0.94	23.7	8.9	16.1	20.4	30.0
L_1t_2	4.9	21.3	4.17	26.1	8.1	12.1	20.6	28.9
L_3t_1	6.2	25.4	0.28	22.2	7.6	19.7	16.8	33.4
L_2t_1	5.7	21.3	0.66	25.5	7.8	17.2	18.3	30.5
L_1t_1	3.5	20.7	3.41	27.9	6.3	14.6	18.3	29.5

INDOOR EXPERIMENT 1963-III

	g dm pot^{-1}	% dm	% NO_3	% cp	% ash	% wsc	% cf	% r
moist treatments								
L_3t_3	51.6	27.8	0.08	8.2	3.6	21.2	27.9	39.0
L_2t_3	40.6	22.1	0.07	10.4	4.6	18.8	28.7	37.4
L_1t_3	24.7	15.2	1.66	18.3	7.3	8.2	29.0	35.5
L_3t_2	38.7	23.9	0.09	8.8	3.9	26.7	23.3	37.2
L_2t_2	37.2	19.7	0.09	11.9	4.5	21.2	26.8	35.5
L_1t_2	21.0	12.7	2.60	20.1	9.0	7.9	27.9	32.5
L_3t_1	33.5	23.4	0.12	11.4	3.9	33.2	19.5	31.9
L_2t_1	29.1	19.6	0.09	13.2	7.6	28.4	21.6	29.1
L_1t_1	18.3	12.6	2.39	22.7	13.3	9.0	25.6	27.0
dry treatments								
L_3t_3	24.0	27.4	0.42	14.8	5.7	16.6	24.1	38.4
L_2t_3	21.0	25.4	1.24	17.0	6.4	17.5	25.1	32.8
L_1t_3	9.9	21.5	5.02	21.8	9.2	12.2	22.3	29.5
L_3t_2	18.2	24.4	0.85	18.6	7.7	17.2	21.4	34.2
L_2t_2	16.2	22.4	2.19	21.0	8.0	16.0	22.0	30.8
L_1t_2	8.9	20.1	5.97	24.4	9.6	12.5	20.8	26.7
L_3t_1	15.9	25.5	0.80	19.9	6.6	21.2	17.6	33.9
L_2t_1	16.6	24.8	0.90	20.7	7.1	24.4	16.6	30.3
L_1t_1	8.8	20.9	4.48	25.2	11.3	15.6	19.0	24.4

INDOOR EXPERIMENT 1963-IV

	g dm pot^{-1}	% dm	% NO_3	% cp	% ash	% wsc	% cf	% r
moist treatments								
L_3t_3	22.1	24.0	0.04	11.5	5.5	20.7	23.9	38.4
L_2t_3	20.7	19.3	0.11	14.0	6.4	15.5	27.8	36.2
L_1t_3	14.2	13.1	2.83	22.7	9.7	4.9	27.8	32.1
L_3t_2	22.3	22.5	0.08	12.1	4.7	26.1	22.0	35.0
L_2t_2	19.5	18.8	0.09	14.2	6.4	18.7	24.7	35.9
L_1t_2	13.5	12.8	1.45	24.2	11.4	7.7	24.1	31.1
L_3t_1	17.8	22.5	0.04	14.2	6.1	27.7	19.4	32.6
L_2t_1	14.7	18.1	0.16	19.2	8.1	17.3	20.3	34.9
L_1t_1	10.3	12.8	4.08	26.8	12.7	5.0	21.6	29.8
dry treatments								
L_3t_3	11.9	23.1	0.38	19.4	11.2	14.4	21.8	32.8
L_2t_3	11.0	20.7	1.34	22.3	12.1	12.5	21.9	30.0
L_1t_3	5.2	17.5	5.22	26.8	9.1	5.3	23.3	30.3
L_3t_2	12.8	22.0	0.37	18.4	10.1	16.1	22.5	32.5
L_2t_2	11.1	19.8	1.46	22.7	11.6	11.8	22.4	30.0
L_1t_2	4.8	19.7	3.16	27.2	10.2	7.0	23.0	29.4
L_3t_1	9.9	22.2	0.50	22.9	7.8	17.7	20.7	30.4
L_2t_1	8.1	19.9	1.49	27.9	8.5	13.4	17.3	31.4
L_1t_1	4.0	19.0	3.95	28.5	12.2	7.9	19.5	27.9

INDOOR EXPERIMENT 1963-V

moist treatments								
L_3t_3	22.1	26.3	0.05	10.8	5.2	25.1	23.9	34.9
L_2t_3	21.8	21.8	0.03	13.3	6.0	20.8	25.1	34.8
L_2t_2	21.9	19.5	0.05	16.3	7.2	18.3	23.1	35.0
L_3t_1	20.8	22.9	0.08	14.5	6.0	26.4	20.3	32.7
L_2t_1	17.9	19.2	0.17	19.1	8.0	17.7	22.9	32.1
dry treatments								
L_3t_3	13.0	26.9	0.71	18.7	8.0	16.6	22.1	33.9
L_2t_3	12.3	24.3	1.04	20.8	8.5	13.9	22.9	32.9
L_2t_2	12.6	24.2	1.21	21.7	8.2	17.0	19.6	32.3
L_3t_1	11.0	26.4	0.62	21.3	8.2	18.9	20.4	30.6
L_3t_1	10.9	25.8	1.24	22.1	8.2	17.7	19.0	31.8

Appendix 2, cont.

INDOOR EXPERIMENT 1963-VI

	g dm pot^{-1}	% dm	% NO_3	% cp	% ash	% wsc	% cf	% r
moist treatments								
L_3t_3	16.5	17.0	1.49	23.5	9.8	5.3	28.3	31.6
L_2t_3	11.2	15.1	5.28	28.6	11.6	2.6	27.9	24.0
L_1t_3	5.0	17.3	7.88	26.7	14.1	0.6	27.4	23.3
L_3t_2	16.8	18.1	0.83	21.3	8.8	11.2	25.3	32.6
L_2t_2	12.6	15.6	3.04	25.7	11.4	6.4	26.4	27.1
L_1t_2	5.6	16.4	6.59	28.0	14.5	0.7	27.0	23.2
L_3t_1	13.2	16.9	1.29	24.9	8.7	10.6	23.7	30.8
L_2t_1	10.5	16.2	3.97	29.0	11.2	5.7	23.0	27.1
L_1t_1	5.5	15.3	5.77	27.8	12.6	2.2	26.9	24.7
dry treatments								
L_3t_3	6.5	26.7	3.76	24.4	10.0	10.5	23.2	28.1
L_2t_3	4.9	26.7	4.73	26.4	11.1	8.6	24.3	24.9
L_1t_3	3.6	30.8	5.46	24.5	11.7	7.1	26.8	24.4
L_3t_2	9.1	22.9	2.16	24.8	9.2	11.4	22.1	30.3
L_2t_2	4.2	28.3	4.18	25.5	10.7	12.3	22.5	24.8
L_1t_2	3.2	29.1	5.93	25.2	12.8	8.0	25.3	22.8
L_3t_1	6.7	27.8	1.86	24.1	8.5	18.3	18.5	28.7
L_2t_1	4.9	26.9	3.18	24.5	9.9	15.1	21.6	25.7
L_1t_1	3.4	27.3	5.23	25.4	11.9	8.1	25.7	23.7

APPENDIX 3

INDOOR EXPERIMENT 1964

	g dm pot^{-1}	% dm	% NO_3	% cp	% ash	% wsc	% cf	% r
L_3N_3	24.3	17.1	0.28	21.7	7.9	18.4	20.1	31.6
L_2N_3	17.9	14.8	1.29	28.5	10.3	11.3	19.7	28.9
L_1N_3	8.5	11.7	5.37	29.4	15.3	3.9	23.9	22.1
L_3N_2	14.3	24.7	0.04	11.2	6.4	33.0	17.2	32.2
L_2N_2	12.0	19.8	0.07	15.5	8.0	26.5	18.6	31.3
L_1N_2	8.3	12.4	2.38	23.3	12.1	7.4	25.1	29.7
L_3N_1	5.0	31.1	0.07	7.2	6.9	38.8	16.5	30.5
L_2N_1	5.8	29.1	0.20	8.6	6.8	38.1	16.0	30.3
L_1N_1	5.7	17.1	0.06	13.0	10.1	20.4	23.3	33.1

APPENDIX 4

FIELD EXPERIMENT, AUTUMN 1964

	kg dm a^{-1}	% dm	% NO_3	% cp	% ash	% wsc	% cf	% r
L_3N_2	42.6	17.2	0.22	17.4	8.0	19.2	20.9	34.3
L_2N_2	31.5	12.3	1.06	24.8	9.7	13.0	20.0	31.4
L_1N_2	21.9	10.6	3.37	24.1	12.0	6.2	25.0	29.3
L_3N_1	24.1	24.2	0.08	10.4	6.4	27.7	20.3	35.1
L_2N_1	22.3	13.8	0.07	15.6	7.6	20.0	21.6	35.1
L_1N_1	18.4	11.2	0.96	21.2	11.1	7.7	24.2	34.8